Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Food Engineering 70 (2005) 546–556
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Pectin methylesterase catalyzed firming effects on low temperature blanched vegetables

Li Ni [a], Daniel Lin, Diane M. Barrett [b,*]

[a] Institute of Biotechnology, Fuzhou University, 350002, China
[b] Department of Food Science and Technology, Cruess Hall, University of California, 1 Shields Avenue, Davis, CA 95616, United States

Received 22 March 2004; accepted 4 October 2004
Available online 18 December 2004

Abstract

The effect of low temperature blanching on firmness of eight vegetables was studied. Preheating vegetables at low temperatures prior to a conventional blanch resulted in firmer products. Temperature and time had significant effects on texture, with temperature the most influential. Under optimal conditions, firmness improvements in preheated vegetables as compared to blanched controls were: Bok choy—3.0 × (65 °C, 45 min); Chinese cabbage—1.8 × (55 °C, 45 min); cabbage—1.6 × (65 °C, 15 min); green bell peppers—1.36 × (70 °C, 15 min); sugar snap peas—1.7 × (65 °C, 30 min); carrots—2.1 × (60 °C, 15 min) and broccoli—2.9 × (60 °C, 15 min). Thermal stability and optimal temperature for pectin methylesterase in homogenates from these vegetables were also analyzed. The relationship between optimum preheating conditions for textural integrity and pectin methylesterase activity is discussed.

Keywords: Blanching, Texture; Pectin methylesterase; Bok choy; Cabbage; Bell peppers; Sugar snap peas; Broccoli; Carrot

1. Introduction

Consumer acceptance of processed vegetables depends on a number of factors, including appearance, texture, flavor and nutritional value. Processed vegetables that maintain firm, crunchy textures are highly desirable because consumers associate these textures with freshness and wholesomeness (Bourne, 2002; Fillion & Kilcast, 2000, 2002; Szczesniak, 1988). Indeed, the appearance of a soft or limp product may cause a lack of acceptance even prior to consumption.

The thermal operations utilized in the production of canned or frozen vegetables frequently result in a significant loss of textural integrity. The most immediate effect of high temperatures is a loss of turgor pressure (Bourne, 1989; Greve et al., 1994; Luna-Guzman & Barrett, in preparation; Ma & Barrett, 2002). As vegetables are heated more, pectins are degraded and solubilized from the cell wall and the middle lamella between adjacent cell walls. This results in a loss of adhesion between cells and a further decrease in tissue firmness (Greve, McArdle, Gohlke, & Labavitch, 1994; Stolle-Smits, Beekhuizen, Recourt, Voragen, & Van Dijk, 1997).

Early studies aimed at maintenance of processed vegetable texture found that preheating vegetables in a long time, low temperature (LTLT) blanch results in a firmer product following subsequent processing at high temperatures (Hoogzand & Doesburg, 1961; Van Buren, Moyer, Wilson, Robinson, & Hand, 1960). This effect has been the subject of numerous studies over the past two decades and has been shown to occur in a multitude of vegetables (Aguilera-Carbo et al., 1999; Bartolome & Hoff, 1972; Chang, Lai, & Chang, 1995; Fuchigami, Miyazaki, & Hyakumoto, 1995; Lee, Bourne, & Van Buren, 1979; Quintero-Ramos, Bourne, Barnard, & Anzaldua-Morales, 1998; Seow, Tan, & Lim, 1991;

* Corresponding author. Tel.: +1 530 752 4800; fax: +1 530 754 7677.
E-mail address: dmbarrett@ucdavis.edu (D.M. Barrett).

0260-8774/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2004.10.009

Stanley, Bourne, Stone, & Wismer, 1995; Steinbuch, 1976; Stolle-Smits, Beekhuizen, Recourt, Voragen, & Dijk, 2000; Wu & Chang, 1990).

The firming effect resulting from a low temperature treatment has been attributed to stimulation of the enzyme pectin methylesterase (PME), which is activated at temperatures between 50 and 70 °C (Chang, Liao, & Wu, 1996; Hou & Chang, 1996; Van Buren, 1979). PME hydrolyzes the methyl ester linkages in pectin molecules, releasing methanol and free galacturonic acid moieties. The resulting free carboxyl groups may then form cross-links between pectin polymers through salt-bridge formation with divalent cations (notably Ca^{2+}) naturally present in the tissues or added to the blanching water.

The de-esterification of pectin by PME during preheating prevents trans-elimination of pectin, thus maintaining the texture of vegetables. Hou and Chang (1996) showed that PME also catalyzed a transacylation reaction of the galacturonic acyl groups from methanol to other hydroxyl groups of pectin, resulting in the formation of new ester linkages between pectin molecules and thus contributing to tissue firming. However, PME may not always be associated with firming. McFeeters, Fleming, and Thompson (1985) found no relationship between PME and cucumber tissue firmness. Hudson and Buescher (1986) have suggested that excessive demethylation may change the pectin configuration and cause softening by the loosening of cell wall components. The relationship between measured PME activity and observed vegetable firmness is complex.

Edible vegetables may be classified into the three primary plant parts from which they are derived: (1) leaves and flowers, (2) fruit (containing seeds) and (3) roots. There is little or no information in the literature on the firming of leafy vegetables by low temperature blanching treatments. Optimal preheating treatments have been evaluated, however, for some rehydrated fruit vegetables, including bell and Jalapeno peppers, snap bean pods and green beans (Howard, Burma, & Wagner, 1997; Huang & Thompson, 1989; Pala, 1982; Quintero-Ramos et al., 1998; Stolle-Smits et al., 2000). There is no published information on the use of LTLT treatments prior to freezing broccoli, peppers or sugar snap peas. In contrast, there has been considerable work on the application of low temperature blanching treatments to carrots (Fuchigami, Hyakumoto, & Miyazaki, 1995; Lee et al., 1979; Lee, Lee, & Lee, 2001; Mohamed & Hussein, 1994). However, optimal LTLT temperatures and times published by these authors ranged widely from 55 to 79 °C and 10 to 60 min, respectively, depending on variety and piece size. Both Tijskens, Waldron, Ng, Ingham, and Dijk (1997) and Verlinden and Baerdemaeker (1997) used mathematical models to describe LTLT effects on carrots, but the former focused on PME activation while the latter studied firming.

The purpose of this project was to evaluate the effects of low temperature blanching treatments on both texture and PME activation in a variety of vegetable tissues. PME activity and texture were measured under a range of preheating conditions and changes in PME activity were correlated with those in vegetable texture. We determined optimal preheating conditions for improving the firmness of eight different vegetables.

2. Materials and methods

2.1. Sample preparation and blanching treatments

The eight vegetables used in this study (Bok choy, Chinese cabbage, cabbage, green and red bell pepper, sugar snap peas, carrots and broccoli) were purchased from a local market. All vegetables were washed and, with the exception of sugar snap peas, which were snipped and used whole, then cut into long, thin slices. Peppers were cut into slices 1 cm (3/8″) wide by 2.5–3.75 cm (1–1½′) in length; all other vegetables were cut into slices 0.6 cm (1/4″) wide and 2.5–3.75 cm (1–1½″) in length. The slice dimensions were determined by the intended commercial application, e.g. they were to be used in a frozen pocket sandwich of specific dimensions. Our experiments were also set up so as to minimize the effects of come up time during the LTLT treatments and blanching.

Blanching treatments were carried out in a water bath which contents were circulated with controlled temperature (Model PC+20B, Julabo USA, Kutztown, PA, USA). When the room temperature vegetable samples were introduced into the rapidly moving water, the temperature dropped 1–2 °C but then returned to the set point within 2 min. Vegetable slices were not restrained, but heated in the bulk liquid to simulate a commercial water blanching operation. The control sample was blanched in water at 100 °C for 3 min, and cooled by immersion in ice water for 1 min. Commercially, these vegetables are typically blanched in 100 °C water for 2.5–3 min and cooled in ambient temperature water. The LTLT samples were first preheated at different temperatures (55, 60, 65, 70, 75, and 80 °C) for varying times (5, 15, 30, 45 and 60 min), then blanched in 100 °C water for 3 min, and cooled by immersion in ice water for 1 min.

2.2. Heat penetration kinetics

Our experiments were set up in such a way that come-up times were minimized. In order to estimate the time required for the different vegetable samples to heat up during the blanching treatment, we used the Heisler model and charts to predict the center temperature of certain geometric shapes as a function of time. We

assumed that there was no significant barrier to heat transfer in the blanching water, but solely inside the vegetable. For this model we need to know the room and initial sample temperatures and the geometric and thermal diffusivity parameters of the vegetable pieces used (Batty & Folkman, 1983). Values of thermal diffusivity that have been reported for vegetables do not vary much for different type of vegetables. For example, reported values for potatoes are $1.3–1.4 \times 10^{-7} m^2/s$ (Magee & Bransburg, 1995) and for onion: $1.2–1.3 \times 10^{-7} m^2/s$ (Rapusas & Driscoll, 1995). For our estimations we used a value of $1.2 \times 10^{-7} m^2/s$ to ensure adequate heating.

The geometrical shape of the vegetables was described as an infinite cylinder of 6×10^{-3} m. We calculated with the Heisler charts that a vegetable piece of these dimensions would heat up from the initial temperature of 20 °C to a center temperature of 69.5 °C in a 70 °C water bath in 1.3 min. Non-cylindrical geometries, like snap peas or pepper pieces can be more appropriately described by a plate shape, which resulted in even smaller come-up times. Based on this analysis we concluded that we can safely assume that there will not be a significant difference in the actual local temperature in the intact pieces used in texture measurements compared to the homogenates used for the PME enzyme assay (see below). We confirmed these come-up times in the laboratory using small gauge thermocouple wires positioned in the geometric center of our slices. These measurements were repeated five times on different slices for each vegetable.

2.3. Texture measurement

A test of compression force was carried out with a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA/Stable Micro Systems, Haslemere, Surrey, UK), using a constant speed of 1 mm/s. Due to the limited capacity of the Texture Analyzer (50 kg load cell), sample weights of the vegetable slices used in the texture analysis ranged from 20 to 45 g. Room temperature samples were placed in a Kramer shear cell. The cell was fitted with five blades, which had been sharpened to a 45° angle to avoid exceeding the maximum load of the instrument. Distance traveled was standardized at 10 mm from the starting position. Peak force was used to quantify the firmness of the samples. Five replicates of each sample were carried out.

2.4. PME activity assay

PME activity was measured using the method described by Kimball (1999). The enzyme was extracted from each vegetable by homogenizing with 2 volumes (v/w) of 0.2 M NaCl for 1 min, stirring for 30 min, then filtering though filter paper (Whatman No. 4). Citrus pectin and sodium chloride were purchased from Sigma. A 0.5 ml aliquot of vegetable filtrate was mixed with 0.25% pectin-salt (0.2 M NaCl) substrate and incubated at 30 °C. The solution was immediately adjusted to pH 7.0 with 0.1 M NaOH. After the initial adjustment, the solution was stirred and 0.1 M NaOH was added quantitatively until pH 7.0 was re-established. Time was measured until the pH of the solution regained pH 7.0. A blank titration was carried out under the same conditions, but 0.5 ml of 0.2 M NaCl solution was used instead of the vegetable filtrate. The net consumption of NaOH was then used to calculate PME activity. PME activity units (PEU) were calculated by the following formula (Kimball, 1999):

$$\begin{aligned}\text{PEU} = {} & (0.1\,\text{M NaOH}) \\ & \times (\text{volume of } 0.1\,\text{M NaOH})/(0.5\,\text{ml sample}) \\ & \times (\text{time in min})\end{aligned}$$

2.5. Heat stability of PME

The heat stability of PME was evaluated on raw homogenates of each vegetable. The same citrus pectin substrate solution as used in the PME assay was held at a specific temperature (45–75 °C) and equilibrated to pH 7.0 for evaluation of heat stability. A 0.5 ml aliquot of vegetable filtrate was added and the pH was immediately adjusted to pH 7.0 with 0.1 M NaOH. Following this initial adjustment, additional aliquots of 0.1 M NaOH were added to maintain pH 7.0. Both aliquot volume and time were recorded for up to around 30 min. A blank titration was carried out under the same conditions, but 0.5 ml volume of 0.2 M NaCl solution was used instead of the vegetable filtrate. The net consumption of NaOH was determined at each temperature and time studied. The data was plotted as volume of NaOH versus time, rather than as PE units, in order to evaluate the stability over a period of 30 min.

2.6. Calcium analysis

Calcium content was determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The raw vegetables were freeze-dried, ground and ashed. The ash was digested in nitric acid and hydrogen peroxide (Sah & Miller, 1992) and analyzed for calcium content. Calcium values were recorded on a dry-weight basis.

2.7. Statistical analysis

The effects of preheating time and temperature on texture were evaluated using a quadratic regression, which was conducted with a SAS program (version

8.0, SAS Institute Inc., 2000). The estimate and value represent the coefficient and significance of factors, respectively. R^2 stands for the correlation between the raw data and estimated values.

3. Results and discussion

3.1. Effects of low temperature blanching on vegetable firmness

The effects of different low temperature blanching treatments on the firmness of eight different blanched vegetables are shown in Fig. 1a–h. All vegetable samples were blanched under standard conditions (100 °C for 3 min), but treated samples received a low temperature blanch aimed at activating PME first prior to the conventional blanch. The conventional blanch served the purpose of inactivating all of the endogenous enzymes prior to freezing, dehydration or another method of relatively low temperature preservation. Due to the variability in the raw material obtained throughout the year, treated samples were compared to a blanched control prepared the same day, which is represented as the zero time in all graphs.

In most cases, the texture of the conventionally blanched control was significantly less firm when compared to samples treated using a combination of LTLT and conventional blanching (Fig. 1a–h). Conventionally blanched samples only retained 1/3–1/2 of their raw texture. Firmness of the vegetables that received a low temperature blanching pretreatment showed between 80 and almost 200% increase in firmness over the blanched control. One notable exception to this phenomenon was red bell peppers, which did not show increased firmness following an LTLT treatment (Fig. 1e).

Statistical values in Tables 1–3 illustrate the effects of low temperature blanch temperature and time on firmness of specific vegetables. Both the estimated coefficients of the effects of temperature, time and their interactions and the probability of finding significance in error are given. The effects of both LTLT temperature and time on firmness showed significant ($P \leqslant 0.05$) effects in all but red peppers; in carrots the values for P were 0.0512 and 0.0561 for temperature and time, respectively. The relatively high P value obtained in carrots may be explained by raw material differences and the high standard deviation of the measured firmness.

Inspection of estimated coefficients of temperature and time provides some insight into the relative importance of each, and whether their effects are negative or positive with respect to vegetable firmness. With the exception of red pepper, the estimated coefficients of temperature were much higher than those for time for all vegetables, indicating that temperature had more impact on vegetable firmness than time. In the case of all the vegetables studied, estimated coefficients of temperature and time were both positive, while the estimated coefficients of the quadratics of time and temperature were negative. This indicates that increasing either factor improved firmness; however either too high a temperature or too long a low temperature blanching treatment would result in the loss of texture.

It is assumed that the firming effects of low temperature blanching treatments relate to PME-catalyzed demethylation of pectin and subsequent incorporation of endogenous calcium. It has generally been reported that PME is activated between 50 and 70 °C (Chang et al., 1996; Hou & Chang, 1996; Van Buren, 1979) and inactivated or destroyed at higher temperatures closer to 80 °C (Bartolome & Hoff, 1972; Steinbuch, 1976). Extension of the LTLT process for too long a time eventually resulted in loss of texture. This may be due to eventual thermal inactivation of PME or heat-induced degradation of the pectin. In the control samples, which were blanched at 100 °C for 3 min, PME was quickly inactivated. Treatment of Bok choy (Fig. 1a) at temperatures ranging from 55 °C to 65 °C resulted in significant enhancement of firmness as either temperature or time was increased up to 45 min. Bok choy treated to LTLT at 65 °C for 45 min showed the highest firmness, which was three times that of the blanched control. Chinese cabbage results (Fig. 1b) were similar to those of Bok choy. When the LTLT time was shorter than 15 min, firmness increased more at either 60 °C or 65 °C; however after 15 min, 55 °C was the optimal temperature.

In the case of cabbage (Fig. 1c), firmness increased quickly during low temperature blanching at 60 °C and 65 °C, but after 5 min, then the rate of firming increased slowly. When LTLT blanching times were increased up to 30 min, firmness declined at all temperatures. One possible reason may be the lower content of calcium in this tissue. The calcium contents of bok choy and Chinese cabbage reported by USDA (http://www.nal.usda.gov) were 105 mg/100 g and 77 mg/100 g, respectively. However, cabbage only contains 47 mg/100 g calcium. Demethylated pectin cannot form cross-links without calcium, thus the LTLT treatment may not result in improved texture. It may be interesting to study the addition of exogenous calcium salts on the firmness of cabbage.

Low temperature blanching improved the texture of the fruit vegetables, green pepper and sugar snap peas; however, there was no significant effect on red peppers (Fig. 1d–f). For the first 5 min of pretreatment of green peppers (Fig. 1d) there was no increase in firmness. The greatest firmness was obtained when green bell peppers were preheated at 70 °C for 15 min, resulting in firmness values 1.36 times that of the control. Dominguez et al. (2001) reported the optimal preheating treatments of rehydrated green bell peppers were 65 °C for 49 min. Differences in the time required in the previous study may

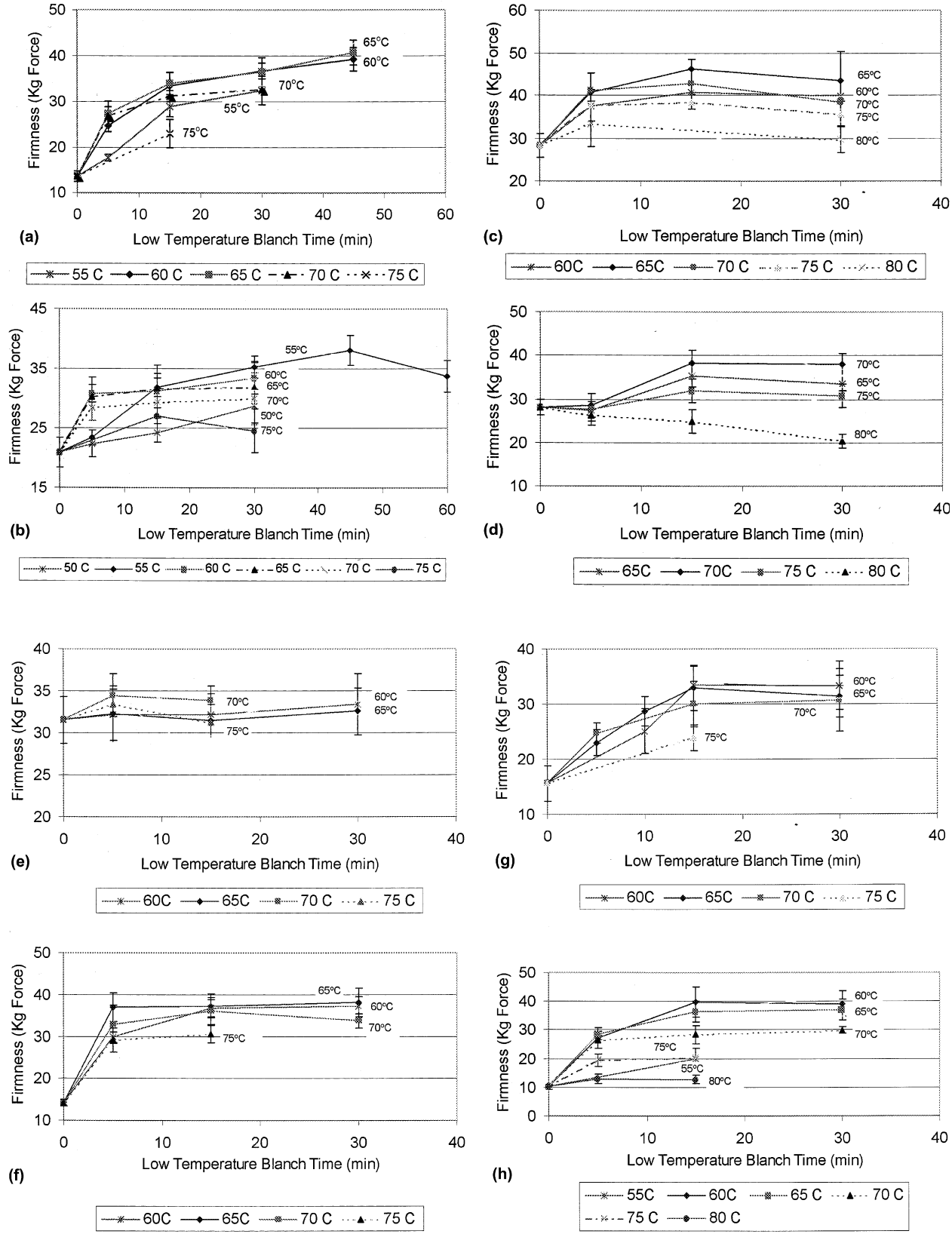

Fig. 1. Firmness of blanched vegetables as affected by low temperature blanching. (a) Bok choy; (b) Chinese cabbage; (c) Cabbage; (d) Green peppers; (e) Red peppers; (f) Sugar snap peas; (g) Carrots; (h) Broccoli.

Table 1
Results of analysis of variance showing effects of LTLT temperature and time on firmness of leafy vegetables

Source	Bok choy		Chinese cabbage		Cabbage	
	Estimate	*P*	Estimate	*P*	Estimate	*P*
Intercept	−347	0.0001	−150	<0.0001	−218	0.0015
Temperature	11.3	<0.0001	5.42	<0.0001	7.51	0.0005
Time	1.83	0.0046	1.48	0.0009	1.45	0.0090
Temperature * temperature	−0.0860	<0.0001	−0.0410	<0.0001	−0.55	0.0004
Time * temperature	−0.0175	0.0446	−0.0183	0.0036	−0.0127	0.0383
Time * time	−0.0846	0.0138	−0.0048	0.0141	−0.0168	0.0211
R^2	0.955		0.871		0.928	

Table 2
Results of analysis of variance showing effects of LTLT temperature and time on firmness of fruit vegetables

Source	Green bell peppers		Red bell peppers		Sugar snap peas	
	Estimate	*P*	Estimate	*P*	Estimate	*P*
Intercept	−457	0.0201	−15.5	0.8028	−334	0.0211
Temperature	13.4	0.0159	1.25	0.5009	10.7	0.0142
Time	3.39	0.0116	1.16	0.1426	2.68	0.0247
Temperature * temperature	−0.0926	0.0156	−0.0078	0.5641	−0.0716	0.0135
Time * temperature	−0.0325	0.0359	−0.0184	0.1075	−0.0280	0.0656
Time * time	−0.0258	0.0398	−0.0003	0.9050	−0.0201	0.0312
R^2	0.894		0.567		0.854	

Table 3
Results of analysis of variance showing effects of LTLT temperature and time on firmness of root/flower vegetables

Source	Carrot		Broccoli	
	Estimate	*P*	Estimate	*P*
Intercept	−327	0.0634	−419	0.0061
Temperature	10.2	0.0512	13.2	0.0031
Time	3.28	0.0561	3.53	0.1029
Temperature * temperature	−0.0757	0.0496	−0.0978	0.0023
Time * temperature	−0.0263	0.1980	−0.0416	0.1268
Time * time	−0.0342	0.0200	−0.0143	0.4102
R^2	0.882		0.879	

be due to the need for rehdyration, whereas our application was on raw green peppers.

Low temperature blanching did not significantly improve the texture of red peppers (Fig. 1e). The primary reason for this may have been that the PME activity in red peppers was too low to be measured (see Discussion below and Table 4). This result confirms that the firming effect of low temperature blanching may be attributed primarily to the action of endogenous PME.

The firmness of sugar snap peas (Fig. 1f) improved rapidly when preheated for 5 min at temperatures ranging from 60 to 75 °C. There was no added benefit of prolonging pretreatment time beyond 15 min.

Preheating carrots at temperatures in the range 60–75 °C also improved textural integrity dramatically (Fig. 1g). This result is consistent with previous studies (Bourne, 1987; Fuchigami et al., 1995; Lee et al., 1979; Lee et al., 2001; Stanley et al., 1995; Tijskens et al., 1997; Verlinden & Baerdemaeker, 1997). When preheated for 15 min at 60 °C and 65 °C, the increase in the firmness was more than two times that of the control.

Lee et al. (1979) reported that the optimum preheating temperature for canned carrots was around 75 °C. This was not the actual carrot temperature; rather this temperature was used to allow for the slow heat

Table 4
PME activities in leafy, fruit, and root/flower vegetables

Leafy vegetables	PEU* (10^{-4})	Fruit vegetables	PEU (10^{-4})	Root/flower vegetables	PEU (10^{-4})
Bok choy midribs	1.11 ± 0.02	Green bell peppers	1.7 ± 0.05	Carrot	2.54 ± 0.11
Bok choy leaves	7.63 ± 0.06	Red bell peppers	0.00	Broccoli	4.32 ± 0.21
Chinese cabbage midribs	0.56 ± 0.02	Sugar snap peas (pods)	157.0 ± 8.4		
Chinese cabbage leaves	2.21 ± 0.07	Sugar snap pea (peas)	33.6 ± 0.64		
Cabbage midribs	1.12 ± 0.01	Sugar snap pea (whole)	178.0 ± 8.19		
Cabbage leaves	1.25 ± 0.05				

* PME activity units (PEU).

penetration into the can. The actual carrot temperature following pretreatment was not determined. Compared to conventional blanching (100 °C for 3 min), Mohamed and Hussein (1994) reported that preheating at 70 °C for 20 min together with calcium treatment could be used to significantly improve the texture of rehydrated dried carrots. However, these authors did not pre-determine the optimal preheating temperature. Quintero-Ramos et al. (1998) studied the effect of low temperature blanching on texture of dehydrated carrots and reported that blanching at 60–65 °C for more than 30 min gave the firmest product after rehydration. Fuchigami et al. (1995) reported that optimum preheating treatments of frozen carrots were at 60 °C for 30 min or 70 °C for 5 min. Our results indicate that carrots preheated at 60 °C or 65 °C for 15 min had firmness values more than twice that of the control.

Broccoli texture (Fig. 1h) was also significantly improved by a low temperature pretreatment when preheating temperatures ranged from 60 to 70 °C. Preheating at 60 °C–70 °C for 5 min, resulted in a similar increase in firmness; however, when time was increased to 15 min, broccoli was firmer after a 60–65 °C treatment. The greatest increase in firmness was obtained when broccoli was preheated at 60 °C for 15 min, after which firmness was 3.9 times that of the control.

3.2. Pectin methylesterase activity in raw vegetables

PME activity in the raw vegetables studied is given in Table 4. Sugar snap peas, and the pods in particular, had by far the highest activity of pectin methylesterase. PME activities in leafy vegetables, green peppers, carrots and broccoli were all in the range of 0.5–7.5×10^{-4} PE units. Leafy vegetables derive most of their texture from the more fibrous midribs, although PME activity was generally higher in leaves. The PME activity in the midribs of Bok choy was double that of Chinese cabbage, which may be the reason why the texture improvement observed in the Bok choy was higher than that in Chinese cabbage after low temperature blanching (Fig. 1a and b). Cabbage had the same level of PME activity as Bok choy; however, as has been stated above, the calcium content in cabbage was definitely lower.

PME activity in green peppers was much lower than that of sugar snap peas, which may explain why firmness increases resulting from low temperature blanching were 2.7 times raw values in sugar snap peas, but only 1.36 times that of raw green pepper. When consumers eat sugar snap peas, the primary perception of crisp texture comes from the pods. It is interesting that PME activity was higher in the pods than in the peas (Table 4). PME activity in sugar snap peas was 100 times that in leafy vegetables; however, the firmness was not improved a hundred fold more by low temperature blanching. Compared to the firmness increase observed in Bok choy and Chinese cabbage, the firmness increase in green peppers was lower although the PME activity of both commodities was similar. Different calcium contents in the tissues of various vegetables may be an important determinant. The firming effect has been postulated to result from increased cross-linking through divalent cations (notably, Ca^{2+}) between free carboxyl groups in pectin molecules. The endogenous calcium content of green peppers and sugar snap peas is only 0.09% and 0.20%, respectively; much lower than that of Bok choy (0.77%) and Chinese cabbage (0.70%).

Carrots and broccoli have somewhat intermediate calcium contents of 0.25% and 0.36%, respectively. Broccoli also has a slightly higher PME activity (Table 4), which may explain why under optimal low temperature blanching conditions broccoli achieved a firmness level 3.9 times that of the blanched control, while the firmest LTLT blanched product for carrots was only 2.1 times that of the control. These results further confirm that the firming effect of low temperature blanching may be attributed to PME, which de-methylates pectin, creating free carboxyl groups that subsequently form calcium cross-links and thereby firm the tissue.

3.3. Effect of temperature on pectin methylesterase activity

The effect of temperatures used for low temperature blanching and time on the PME activity in vegetable homogenates is illustrated in Fig. 2a–h. In most cases, the optimum temperature for PME activity in the vegetable homogenates evaluated was lower than the apparent optimum temperature for firming the same vegetable pieces (Fig. 1a–h and Table 5).

At the beginning of the heating process, PME activity in Bok choy midribs was slightly higher from 50 to 65 °C than at 45 °C (Fig. 2a). This result is consistent with published data, which stated that PME could be activated between 50 and 70 °C (Van Buren, 1979). As heating time proceeded, the rate of PME activity at 50 °C was quite steady, while at temperatures above 55 °C activity decreased significantly. At temperatures of 65 °C, PME was essentially inactivated in 30 min.

The thermal stability of PME in Chinese cabbage midribs was greater than that in Bok choy (Fig. 2b). Even when the cabbage extract was maintained at 60 or 65 °C for 30 min, there was still measurable PME activity. As with Bok choy, PME activity was highest at 50 °C when the extract was held for more than 15 min.

The thermal stability of cabbage PME in the midribs and leaves was very similar (Fig. 2c and d). PME activities at 50 and 55 °C were comparable; however the latter decreased after being held about 20 min. When extracts of cabbage homogenates were held at 60 °C, PME activ-

ity was initially higher than that at 50 °C and 55 °C; however, it became much lower as the heating process proceeded. When extracts were maintained at 65 °C, PME activity was lost quite quickly, which indicated rapid denaturation at this temperature.

After preheating for 30 min, PME generally showed the highest activity in the three leafy vegetables at 50 °C, a temperature lower than the optimum preheating temperature reported in the literature. A few published papers (Hou & Chang, 1996; Puri, Solomos, & Kramer, 1982; Stolle-Smits et al., 2000; Wu & Chang, 1990) reported that the optimum temperature for PME activity in some vegetables (e.g. pea sprouts, potatoes, green beans and stem vegetables) was similar to the optimum temperature we obtained in the LTLT portion of this study on vegetable slice firmness. However, some of these authors have not taken low temperature blanch time into consideration. Our results illustrate that time was also a significant factor in the determination of firmness (Table 2).

In contrast to the leafy vegetables, PME activity in green peppers (Fig. 2e) was quite stable for the first 20 min at temperatures in the range of 65–75 °C. As heating time increased, the greatest thermal stability was observed at 60–65 °C.

PME activity in the other fruit vegetable studied, sugar snap peas, was quite the opposite in that it was very heat labile (Fig. 2f). Even at relatively low temperatures of 50–55 °C, activity levels decreased quite quickly as time increased to more than 20 min. At temperatures greater than 60 °C, PME activity decreased dramatically.

Although PME activity was quite high in the first 5 min of heating carrot extracts at 60–65 °C, activity at this temperature declined rapidly. Thermal stability of the enzyme was much greater at temperatures in the range of 50–55 °C. Similar results were obtained by Chinnery (1983), who reported on the heat sensitivity of carrot PME. At 60 °C, this author found considerable activity was still detectable after 45 min; at 70 °C there was appreciable loss of activity after 15 min, and at 80 °C the enzyme was rapidly inactivated.

Tijskens et al. (1997) found that there were two PME isozymes in carrots; one is bound while the other is reportedly free. They found that as temperatures were increased above 70 °C, the entire bound enzyme was converted to free enzyme, thereby inducing the apparent increase in the rate of denaturation. However, our results showed (Fig. 1g) that even at temperatures in the range of 60–70 °C, at which PME denaturation should

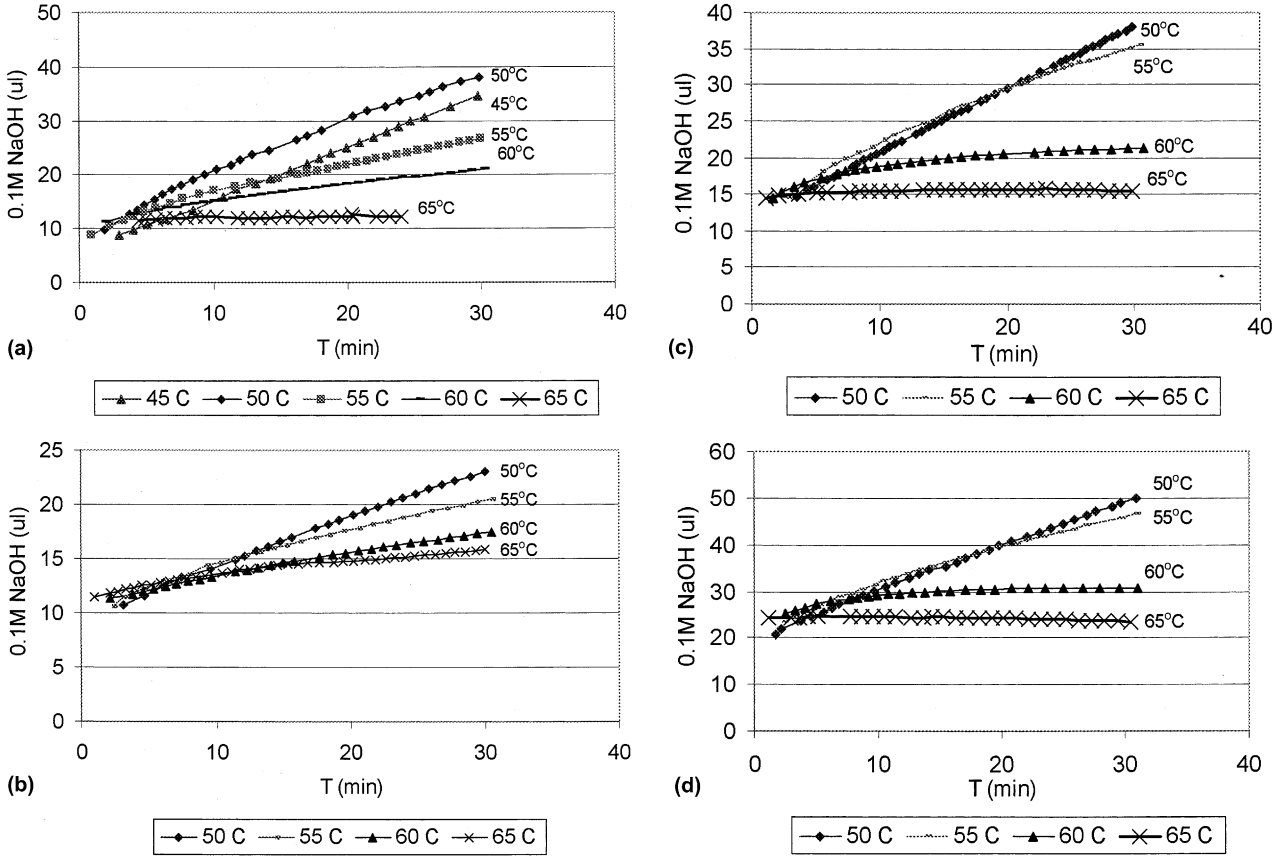

Fig. 2. Effect of temperature on PME activity in various vegetables. (a) Bok choy; (b) Chinese cabbage; (c) Cabbage midribs; (d) Cabbage leaves; (e) Green peppers; (f) Sugar snap peas; (g) Carrots; (h) Broccoli.

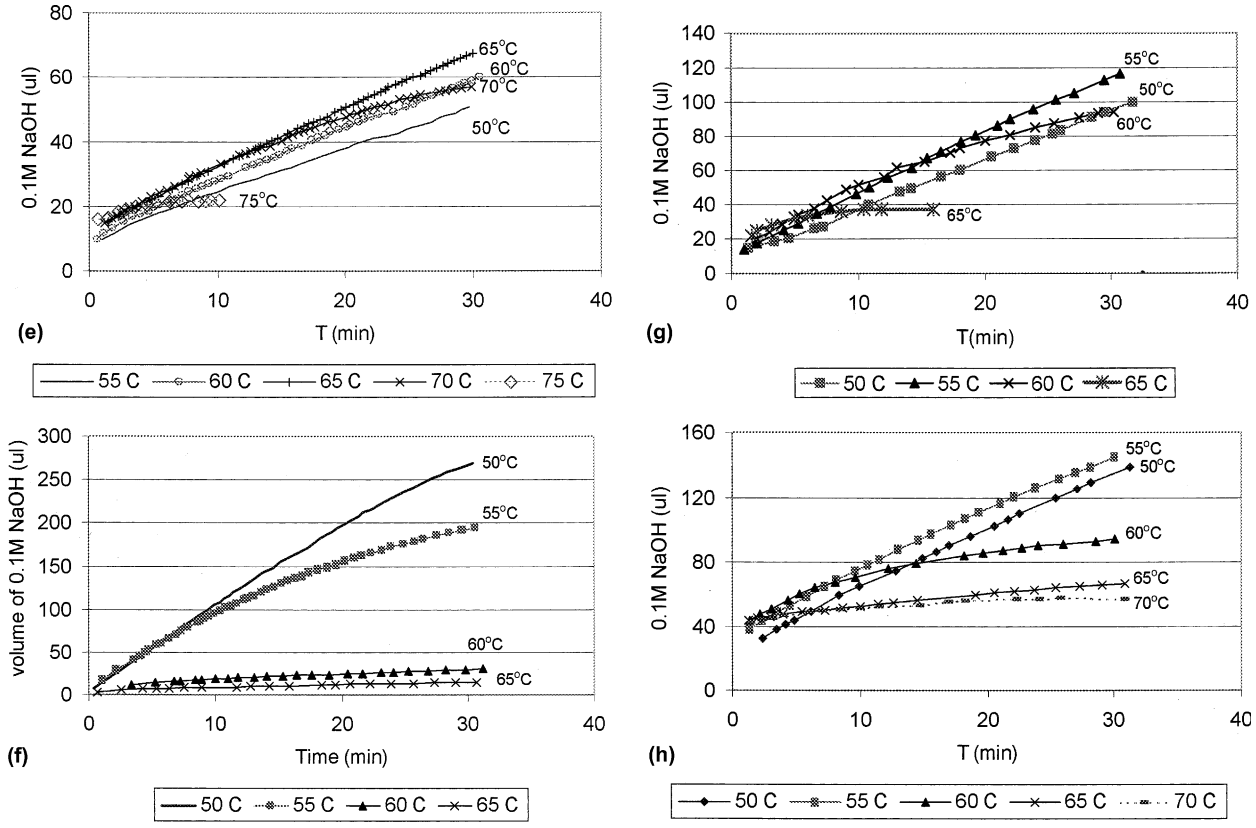

Fig. 2 (*continued*)

Table 5
Temperature optimums for firmness and PME activity in various vegetables

Vegetable	T_{opt} for firmness (°C)	T_{opt} for PME activity (°C)
Bok choy	65	50
Chinese cabbage	55	50
Cabbage	65	50
Green bell peppers	70	65
Sugar snap peas	65	50
Carrots	60	55
Broccoli	60	50–55

have occurred, the firmness of carrot pieces could be significantly improved. Our results may be explained by assuming that although PME activity is ultimately reduced at higher temperatures, initial activity is high enough to form sufficient demethyled pectin, which then cross-links with cations to form firmer tissue. Lee et al. (2001) determined that the optimum temperature for firming carrots was 50 °C, which is lower than the 60–65 °C we determined above.

Broccoli PME showed higher activity at 55 °C than at 50 °C in the first 40 min, after which activity was similar (Fig. 2h). Although initially activity was higher, when maintained at temperatures in the range from 65 to 70 °C, PME appears to be rapidly inactivated after about 5 min. Earlier results (Fig. 1h) indicated that the firmest broccoli could be obtained when florets were pre-maintained at 60 °C or 65 °C for 15 min; however the greatest PME activity was found at 55 °C. As with other vegetable slices, one possible reason for differences in optimal temperature is that heat penetration into stems was slower.

In all of the vegetables studied, with the exception of red peppers in which PME activity was low, the optimal temperature for tissue firming was much higher than that for PME activation (Table 5). These differences may be due in part to the fact that the texture studies were carried out on intact vegetable tissues while the PME activation studies were done on enzyme extracts. There are most likely physical and chemical components of the natural plant system which affect PME-catalyzed firming of the tissue. In the intact tissue, PME is thought to be associated with the cell wall, and therefore may be somewhat protected while the extracted enzyme will be much more heat sensitive.

Puri et al. (1982) also pointed out that PME action is a preliminary step in a process that would yield a substrate for the action of polygalacturonase (PG). PG is one of the primary contributors to vegetable softness. At the optimum temperature for PME, PG may also have relatively high activity and may cause significant softening. Therefore, heating the intact vegetable pieces

may result both in PME-catalyzed firming and a simultaneous increase in PG-catalyzed softening. Choosing temperatures for firming vegetables will require selection of conditions where this combined effect is optimized.

In many vegetables, prolonging low temperature blanching time did not result in additional firming, which may be the result of two factors. First, available calcium ions for cross-link formation with adjacent pectin molecules may have been consumed. Some researchers have confirmed that addition of exogenous calcium salts may improve the firmness in some vegetables (Dominguez et al., 2001; Gu, Howard, & Wagner, 1999; Lee et al., 2001; Stanley et al., 1995). Secondly, as Puri et al. (1982) pointed out, PME provides a substrate for the action of polygalacturonase, whose activity results in hydrolysis of galacturonic acid residues in the pectin polymer and subsequent loss of firmness. To better understand the relationship between observed PME activity and the firming effect induced by low temperature blanching treatments, further studies will need to be conducted.

4. Conclusion

Low temperature blanching treatments of as little as 5–15 min, applied prior to blanching, significantly improved the texture of a number of leafy, bud, fruit and root vegetables as compared to conventional blanching alone. Both low temperature blanching temperature and time have a great impact on firmness. The firming effect is related to the de-esterification of pectin by the action of pectin methylesterase. Optimal temperatures for PME activity in a vegetable homogenate were typically lower than those for vegetable tissue firming. Further investigation should be carried out to determine the causal relationship between observed PME activity and tissue firming.

Acknowledgement

We would like to acknowledge the generous support of Uncle Ben's Inc. (a division of M&M Mars Inc.) for this project, and the enthusiastic collaboration of Ms. Cindy Kratochvil in particular. In addition, we would like to thank Dr. Matthijs Dekker of Wageningen University, The Netherlands, for his assistance with the heat penetration kinetics.

References

Aguilera-Carbo, A., Montanez, J. C., Anzaldua-Morales, A., de la Luz Reyes, M., Contreras-Esquivel, J., & Aguilar, C. N. (1999). Improvement of color and limpness of fried potatoes by in situ pectinesterase activation. *European Food Research and Technology, 210*, 49–52.

Bartolome, L. G., & Hoff, J. E. (1972). Firming of potatoes: Biochemical effects of preheating. *Journal of Agricultural and Food Chemistry, 20*, 266–270.

Batty, J. C., & Folkman, S. L. (1983). *Food engineering fundamentals*. New York, NY: John Wiley & Sons.

Bourne, M. (2002). *Food texture and viscosity* (2nd ed.). *Food Science and Technology, International Series*. San Diego, CA: Academic Press.

Bourne, M. C. (1987). Effect of blanch temperature on kinetics of thermal softening of carrots and green beans. *Journal of Food Science, 52*, 667–668, 690.

Bourne, M. C. (1989). Applications of chemical kinetic theory to the rate of thermal softening of vegetable tissue. In J. Jen (Ed.), *Quality factors of fruits and vegetables: Chemistry and Technology. American Chemical Society Symposium Series No. 415* (pp. 98–110). Washington, DC: ACS.

Chang, C. Y., Lai, L. R., & Chang, W. H. (1995). Relationships between textural changes and the changes in linkages of pectin substances of sweet pepper during cooking processes and the applicability of the models of interactions between pectin molecules. *Food Chemistry, 53*, 409–416.

Chang, C. Y., Liao, H. J., & Wu, T. P. (1996). Relationships between the textural changes and the contents of calcium, magnesium ions, and non-freezing water in the alcohol-insoluble solids of snap bean pods during cooking processes. *Food Chemistry, 55*(1), 49–53.

Chinnery, L. M. (1983). Pectin methylesterase activity and the texture of carrot slices cooled in an electric casserole. *Journal of Consumer Studies and Home Economics, 7*, 109–116.

Dominguez, R., Quintero-Ramos, A., Bourne, M. C., Barnard, J., Talmas-Abbud, R., Jimenez-Castro, J., & Anzaldua-Morales, A. (2001). Texture of rehydrated dried bell peppers modified by low-temperature blanching and calcium addition. *International Journal of Food Science and Technology, 36*, 523–527.

Fillion, L., & Kilcast, D. (2000). Concept and measurement of freshness of fruits and vegetables. Leatherhead Food RA Research Reports No. 770.

Fillion, L., & Kilcast, D. (2002). Consumer perception of crispness and crunchiness in fruits and vegetables. *Food Quality and Preference, 13*, 23–29.

Fuchigami, M., Hyakumoto, N., & Miyazaki, K. (1995). Programmed freezing affects texture, pectic composition and electron microscopic structures of carrots. *Journal of Food Science, 60*, 137–141.

Fuchigami, M., Miyazaki, K., & Hyakumoto, N. (1995). Frozen carrot texture and pectic components as affected by low-temperature-blanching and quick freezing. *Journal of Food Science, 60*(1), 132–136.

Greve, L. C., McArdle, R. N., Gohlke, J., & Labavitch, J. M. (1994). The impact of heating on carrot firmness: Changes in cell wall components. *Journal of Agricultural and Food Chemistry, 42*, 2900–2906.

Greve, L. C., Shackel, K. A., Ahmadi, H., McArdle, R. N., Gohlke, J., & Labavitch, J. M. (1994). The impact of heating on carrot firmness: the contribution of cellular turgor. *Journal of Agricultural and Food Chemistry, 42*, 2896–2899.

Gu, Y. S., Howard, L. R., & Wagner, A. B. (1999). Firmness and cell wall characteristics of pasteurized jalapeno pepper rings as affected by calcium chloride and rotary processing. *Journal of Food Science, 64*(3), 494–497.

Hoogzand, C., & Doesburg, J. J. (1961). Effect of blanching on texture and pectin of canned cauliflower. *Food Technology, 15*, 160–163.

Hou, W. C., & Chang, W. H. (1996). Pectinesterase-catalyzed firming effects during precooking of vegetables. *Journal of Food Biochemistry, 20*(5), 397–416.

Howard, L. R., Burma, P., & Wagner, A. B. (1997). Firmness and cell wall characteristics of pasteurized jalapeno pepper rings as affected

by preheating and storage. *Journal of Food Science, 62*(1), 89–92, 112.

Huang, Y.-C., & Thompson, D. R. (1989). Changes in texture of green peas during freezing and frozen storage. *Journal of Food Science, 54*(1), 96–101.

Hudson, J. M., & Buescher, R. W. (1986). Relationship between degree of pectin methylation and tissue firmness of cucumber pickles. *Journal of Food Science, 51*(1), 138–140, 149.

Kimball, D. A. (1999). *Citrus processing: a complete guide* (2nd ed.). Gaithersburg, MD: Aspen Publishers, Inc., p. 450.

Lee, C. Y., Bourne, M. C., & Van Buren, J. P. (1979). Effect of blanching treatments on the firmness of carrots. *Journal of Food Science, 44*, 615–616.

Lee, G. H., Lee, S. K., & Lee, S.-H. (2001). Characterization of pectionlytic enzyme and blanching condition of raw carrots. *Journal of Korean Society for Food Science and Nutrition, 30*(2), 228–233.

Luna-Guzman, I., & Barrett, D. M., (in preparation). Turgor pressure component of calcium-induced firming of tomato (*Lycopersicum esculentum*) pericarp tissue.

Ma, W., & Barrett, D. M. (2002). Effects of maturity and processing variables on heat penetration times, firmness and drained weight of diced tomatoes (Halley Bos 3155 cv). *Journal of Food Processing and Preservation, 26*, 75–89.

Magee, T. R. A., & Bransburg, T. (1995). Measurement of thermal diffusivity of potato, malt bread and wheat flour. *Journal of Food Engineering, 25*(2), 223–232.

Mohamed, S., & Hussein, R. (1994). Effect of low temperature blanching, cysteine-HCl, *N*-Scetyl-L-Cysteine, Na Metabisulphite and dring temperatures on the firmness and nutrient content of dried carrots. *Journal of Food Processing and Preservation, 18*, 343–348.

McFeeters, R. F., Fleming, H. P., & Thompson, R. L. (1985). Pectinesterase activity, pectin methylation, and texture changes during storage of blanched cucumber slices. *Journal of Food Science, 50*, 201–205, 219.

Pala, E. (1982). Effect of different pretreatments on the quality of deep frozen green beans and carrots. *Refrigeration Science and Technology, 4*, 224–231.

Puri, A., Solomos, T., & Kramer, A. (1982). Partial purification and characterization of potato pectinesterase. *Food Chemistry, 8*, 203–213.

Quintero-Ramos, A., Bourne, M. C., Barnard, J., & Anzaldua-Morales, A. (1998). Optimization of low temperature blanching of frozen jalapeno pepper using response surface methodology. *Journal of Food Science, 63*(3), 519–522.

Rapusas, R. S., & Driscoll, R. H. (1995). Thermophysical properties of fresh and dried white onion slices. *Journal of Food Engineering, 24*, 149–164.

Sah, R. N., & Miller, R. O. (1992). Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Analytical Chemistry, 64*, 230–233.

Seow, C. C., Tan, S. C., & Lim, S. J. (1991). Production of firmer-textured canned and frozen tropical fruit and vegetables. *ASEAN Food Journal, 6*(3), 104–108.

Stanley, D. W., Bourne, M. C., Stone, A. P., & Wismer, W. V. (1995). Low temperature blanching effects on chemistry, firmness and structure of canned green beans and carrots. *Journal of Food Science, 60*, 327–333.

Steinbuch, E. (1976). Technical note: Improvement of texture of frozen vegetables by stepwise blanching treatment. *Journal of Food Technology, 11*, 313–315.

Stolle-Smits, T., Beekhuizen, J. G., Recourt, K., Voragen, A. G. J., & Van Dijk, C. (1997). Changes in pectic and hemicellulosic polymers of green beans (*Phaseolus vulgaris* L.) during industrial processing. *Journal of Agricultural and Food Chemistry, 45*, 4790–4799.

Stolle-Smits, T., Beekhuizen, G., Recourt, K., Voragen, A. G. J., & Dijk, V. C. (2000). Preheating effects on the textural strength of canned green beans. 1. Cell wall chemistry. *Journal of Agricultural and Food Chemistry, 48*, 5269–5277.

Szczesniak, A. S. (1988). The meaning of textural characteristics—crispness. *Journal of Texture Studies, 19*, 51–59.

Tijskens, L. M. M., Waldron, K. W., Ng, A., Ingham, L., & Dijk, C. V. (1997). The kinetics of pectin methyl esterase in potatoes and carrots during blanching. *Journal of Food Engineering, 34*, 371–385.

USDA website http://www.nal.usda.gov/fnic/foodcomp/.

Van Buren, J. P. (1979). The chemistry of texture in fruits and vegetables. *Journal of Text Studies, 10*, 1–23.

Van Buren, J. P., Moyer, J. C., Wilson, D., Robinson, W. B., & Hand, D. B. (1960). Influence of blanching conditions on sloughing, splitting, and firmness of canned snap beans. *Food Technology, 14*, 233–236.

Verlinden, B. E., & Baerdemaeker, J. D. (1997). Modeling low temperature blanched carrot firmness based on heat induced processes and enzyme activity. *Journal of Food Science, 62*(2), 213–218.

Wu, A., & Chang, W. H. (1990). Influence of precooking on the firmness and pectic substances of three stem vegetables. *International Journal of Food Science and Technology, 25*, 558–565.