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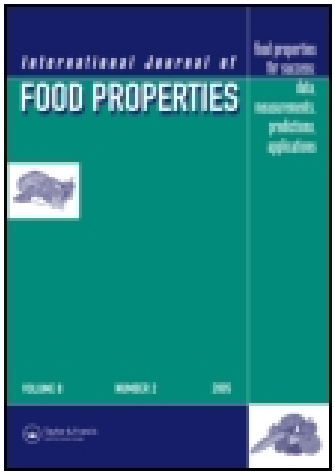

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SEASONALITY OF THE THERMAL KINETICS OF COLOR CHANGES IN WHOLE SPINACH (*SPINACIA OLERACEA*) LEAVES UNDER PASTEURIZATION CONDITIONS

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Color changes in whole spinach (*Spinacia oleracea*) leaves at pasteurization temperatures (65 to 90°C) indicate that the parameter of "greenness" ($-a_t/b_t$) increased during a short initial period of heating, followed by a loss that was more pronounced at higher temperatures. Seasonality was evident in kinetic models for color changes possibly due to seasonal difference in chemical composition influencing color degradation kinetics. The mechanism for loss of greenness at lower temperatures was attributed to enzymatic activity while cell collapse, cell compaction, and oxidative changes were probably more important at higher temperatures. Lower temperatures resulted in a higher retention of green color of spinach leaves during the thermal pasteurization process and the kinetic models presented in this work could be used for optimizing pasteurization processes.

Keywords: ***Thermal processing, Reaction kinetics, Browning index, Cell structure, Scanning electron microscopy.***

INTRODUCTION

Spinach (*Spinacia oleracea*) is an important cool annual leafy vegetable grown throughout temperate regions of the world for the fresh market and for processing. Production of fresh spinach in the USA reached 278,190 metric tons in 2010, of which roughly 77,240 metric tons was processed, most of it frozen, with lesser amounts thermally processed and dehydrated.[1] Spinach is rich in iron, potassium, calcium, and vitamin *C*.[2]

While thermal processing is intended to destroy microbes of public health significance, the application of heat to fragile leave vegetables like spinach can cause severe quality deterioration, such as degradation in color and texture, nutrient loss, cook loss, and area shrinkage. Furthermore, consumers require that thermally processed food retain nutritive features and fresh-like quality.[3–6] The color of foods is one of the most important quality factors for vegetables and plays a considerable role in the overall acceptability of

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foods. Color is a component of total appearance and incorporates visual recognition and assessment of the surface and subsurface properties.[2,7] Instrumental color measurement provides an indication of quality and tristimulus colorimetry is well established as a rapid and simple instrumental method to predict the visual perception of foods.[8] Color is most commonly represented in terms of *L, a*, and *b* values (brightness, green to red, blue to yellow, respectively) or a combination of these three parameters depending upon the nature of the pigment in the food material[2,7–12] and optical properties. Other parameters are derived from Hunter *L, a,* and *b* values, such as the total color change (ΔE).

One of the most important parameters in quality assessment for vegetables is the degree of greenness. This reflects changes to chlorophyll that occur during cooking and commercial sterilization processes (80–145°C, 8–5 min).[13] For pasteurization, an increase in green color has been observed during the initial stage of heating in broccoli (40–96°C, 180–4 min)[11] and during the blanching of spinach and mustard greens[8,12] at the ranges of 75 to 115°C and 50 to 120°C, respectively. Heat-induced color changes (from bright green to olive brown) are attributed to conversion of chlorophyll *a* and *b* to their respective pheophytins and further degradation to pyropheophytins.[11,14] Upon prolonged heating, pheophytin is formed through an exchange of Mg^{2+} with H^+ in the center of the porphyrin ring of chlorophyll.[11] Others have shown that instrumental color measurements compare well with chemical determination of chlorophyll loss in heated broccoli juice and provides the advantage of a useful quality assessment that more closely reflects consumer perceptions since green color and visual appearance are more important for product preference than residual chlorophyll content.[15]

Thermally induced loss of quality including color can be predicted from kinetic models, usually first-order, and a number of different models have been developed for pigment and color degradation in fruits and vegetables, such as broccoli,[11,15] peach,[16] peas,[14,17] and chili,[18] leafy green vegetables.[8,12,19–21] Recent studies of color and chlorophyll degradation kinetics in green peas under different thermal temperatures (70 to 100°C)[22] showed that both chlorophyll and color decreased with heating time and that there was greater chlorophyll degradation and color loss with increasing temperature, as anticipated. Most studies on color change from heat treatments only mention a decrease of green color.[11] Only a few researchers mention an initial increase in green color upon heating. For example, Lau et al.[23] noticed an increase in green color of green asparagus during the initial stages of heating between 70 to 98°C. Tijskens et al.[11] also reported that the change in green color due to the heat treatment (40 to 96°C) of broccoli and green beans consisted initially of an increase in color followed by a decrease. Failure to detect this phenomenon in earlier studies was due in part to the time points selected for monitoring the heat treatment. Most studies focus on prolonged heating at higher temperatures. In many of these cases, the vegetables were already blanched before color measurements were performed.

Surprisingly, relatively few studies have been conducted on intact plant tissues, such as whole spinach leaves. Dadali et al.[2,21,24] studied changes in color and moisture diffusivity in whole leaf spinach and okra during microwave dehydration and is one of the few studies on whole tissue systems. Visual changes in green color and the kinetics of color change in spinach puree under different temperature treatments, such as 50 to 100°C for 20–60 min[12] and 75 to 115°C for up to 20 min,[8] showed a predictable and consistent loss in color. We hypothesize that the degree and rate of color changes will be different in whole tissue compared to a puree and that color can be preserved during mild pasteurization

treatments that are sufficient to inactivate pathogens, but which limit tissue damage allowing for the retention of maximal fresh-like color and texture. The objectives of this study were to determine the kinetic of color degradation of whole spinach leaves at pasteurization temperatures noting that there are visual differences in spinach harvested at different times of the year and that the seasonality of color changes may be important for optimizing pasteurization protocols.

MATERIAL AND METHODS

Material

Packaged fresh baby spinach leaves were purchased from a local retailer in Pullman, WA, USA at different periods over one calendar year, and transferred to the School of Food Science at WSU (Pullman, WA, USA) and stored at 4°C and used within 1 day for the experiments.

Thermal Treatments

Thermal treatments were conducted at 65, 70, 75, 80, 85, and 90°C for various times (Table 1) except for summer, which has been run at 65 to 85°C. All experiments were repeated three times with three replicates ($N = 3$). Treatments were selected to provide sufficient heating to inactivate norovirus and *Listeria monocytogenes*.[25–27] The heating method of Kong et al.[3] was used for whole spinach leaves. Briefly, a single leaf (28 ± 2 mg in wet weight) was hermetically sealed into a custom built cylindrical aluminum test cell having an inner diameter of 35 mm, inner height of 6 mm, and a wall thickness of 2 mm. Come-up time, defined as the time for the sample center temperature to reach within the 1°C of the total temperature rise, was determined using a 0.1-mm diameter copper-constantan thermocouple (Type-T) inserted through the rubber gland in the lid of the container. The immersion length of the probe was 3 mm, thus the influence of heat conduction along the thin wire probe to the sample temperature measurement was considered to be minimal. Distilled water was added to the test cells to cover the leaves (5 ml), and then the cells were sealed and heated in an oil bath (Model HAAKE W13, Thermo Electron Corp., Karlsruhe, Germany) at the specified temperatures using ethylene glycol as the heating medium. After heating, the sample cells were immersed into the mixed ice and water immediately to cool. After cooling, the spinach leaves were dried with a filter paper and color measurements taken.

Table 1 Experimental conditions.

Temperature (°C)	Heating time (min)									
65	1	3	5	7	10	13	23	33	48	63
70	1	3	5	7	11	19	27	35	43	
75	1	2	4	7	10	12	14	16	18	20
80	1	3	5	6	9	12	15	18		
85	1	2	4	5	6	7	9	11	13	
90	1	2	3	4	5	7	9	12		

Color Measurement

Color was measured before and after heat treatments using a Hunter colorimeter (CM-2002, MINOLTA, Osaka, Japan). This system uses three values (*L, a, b*) to describe the precise location of a color inside a three-dimensional visible color space. The colorimeter was calibrated against standard white ($L = 96.72$, $a = 0.11$, $b = -0.14$) and green plates ($L = 65.99$, $a = -18.77$, $b = 9.36$) before a set of color measurement was taken. For each leaf, three measurements were performed. The total color change (ΔE), and greenness (Eqs. 1 and 2, respectively)[2,28] were also calculated from the Hunter *L, a*, and *b* scale to describe the color changes occurring during thermal processing:

$$\Delta E = \sqrt{(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)^2}, \tag{1}$$

where L_0, a_0, and b_0 are the initial color measurements of raw spinach samples and L_t, a_t, and b_t are the color measurements following the thermal treatment times specified above.

$$\text{Greenness} = \frac{-a_t}{b_t}. \tag{2}$$

Color Degradation Kinetics

Generally, reaction rates for color degradation under isothermal conditions can be presented as follows:[2,3,7,12,18,29]

$$\frac{dC}{dt} = -k(C)^n, \tag{3}$$

where k is the rate constant, C is the color at time t, and n is the order of reaction. To find the best empirical relationship, color data were analyzed using zero-, first-, and second-order kinetic models in Eqs. (4)–(6):

$$\text{zero} - \text{order}: C_t = C_o - k.t, \tag{4}$$

$$\text{first} - \text{order}: \text{In}\frac{c_t}{c_o} = -k.t, \tag{5}$$

$$\text{second} - \text{order}: k_t = \frac{1}{c_t} - \frac{1}{c_o}, \tag{6}$$

where C_0 is the initial value of the color at time zero, C_t is the value at time t, and k is the rate constant. Arrhenius equation was used to determine the degradation rate constant (k) on temperature, which is described as follows:

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right), \tag{7}$$

where E_a is the activation energy of the reaction (kJ mol^{-1}), R is universal gas constant (8.3145 J mol^{-1} K^{-1}), T is absolute temperature (K), and k_0 is frequency factor (min^{-1}). If Eq. (7) applies to a reaction in consideration, a plot of the rate constant on semi-logarithmic scale as a function of reciprocal absolute temperature (T^{-1}) should yield a

straight line, and the activation energy can be determined as the slope of the line multiplied by the gas constant R. The R^2 values were used to select the best fit equation.

Scanning Electron Microscopy (SEM)

Cell damage from heating was examined using SEM at 65, 75, and 85°C (representing lower, medium, and higher treatment temperatures) ($N = 2$). Visual differences between micrographs for fresh spinach and spinach subjected to thermal treatments were determined. Spinach leaves after heat treatments were kept at 4°C for 2 h, and immediately transferred to the SEM lab. In the SEM lab, the leaves were cut and then fixed in fixative solution, including 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.1 M phosphate buffer (PBS), and kept at 4°C. Then after rinsing with PBS two times (10 min in each) and then deionized water two times (5 min in each), they were then examined by SEM (Hitachi S-570, Hitachi Ltd., Tokyo, Japan) using an accelerating voltage of 20 KV). Micrographs were taken at the magnification of 500× for transverse section.

Statistical Analysis

The experimental results are presented as mean ± standard deviation of triplicate experiments ($N = 3$).

RESULTS AND DISCUSSION

Visual Color Change in Spinach

The results of visual color change in whole spinach leaves during the pasteurization, showed that with increasing time and temperature the spinach color tended to darken (Fig. 1). Increased shrinkage occurred with increasing time and temperature.

Modeling Color Change in Spinach

The change in visual greenness in winter samples as an indication of changes in chlorophyll pigment content is presented by the ratio of a_t to b_t ($-a_t/b_t$) (Fig. 2). Greenness increased during the initial heating period (1–13 min depending upon temperature). At higher temperatures, a greater increase in greenness could be observed, followed by a rapid loss at longer treatment times. This phenomenon was less pronounced at lower treatment temperatures as observed for peas and string bean,[30] peas,[31] asparagus,[23] green beans, and broccoli.[11] Lau et al.[23] noticed an initial increase in green color of green asparagus at a heating time of 70 to 98°C. Tijskens et al.[11] reported an increase in green color in green beans with loss of greenness upon further heat treatment. However, the chemical and physical factors associated with this change in color are not well understood. Blanching can decrease the opacity of cells altering their optical properties through replacement of intercellular air with blanching water followed by the release of cellular liquids as cell membranes deteriorate.[11,30] In addition, in fresh produce, colorless or weakly colored green precursors that are converted into visible green components would increase color intensity during blanching treatments as chlorophyll degrades.[28,32–34]

Loss of cellular integrity during heat treatment, including damage to cell membrane, permits interaction of enzyme (chlorophyllase) and chlorophyll precursor compounds.

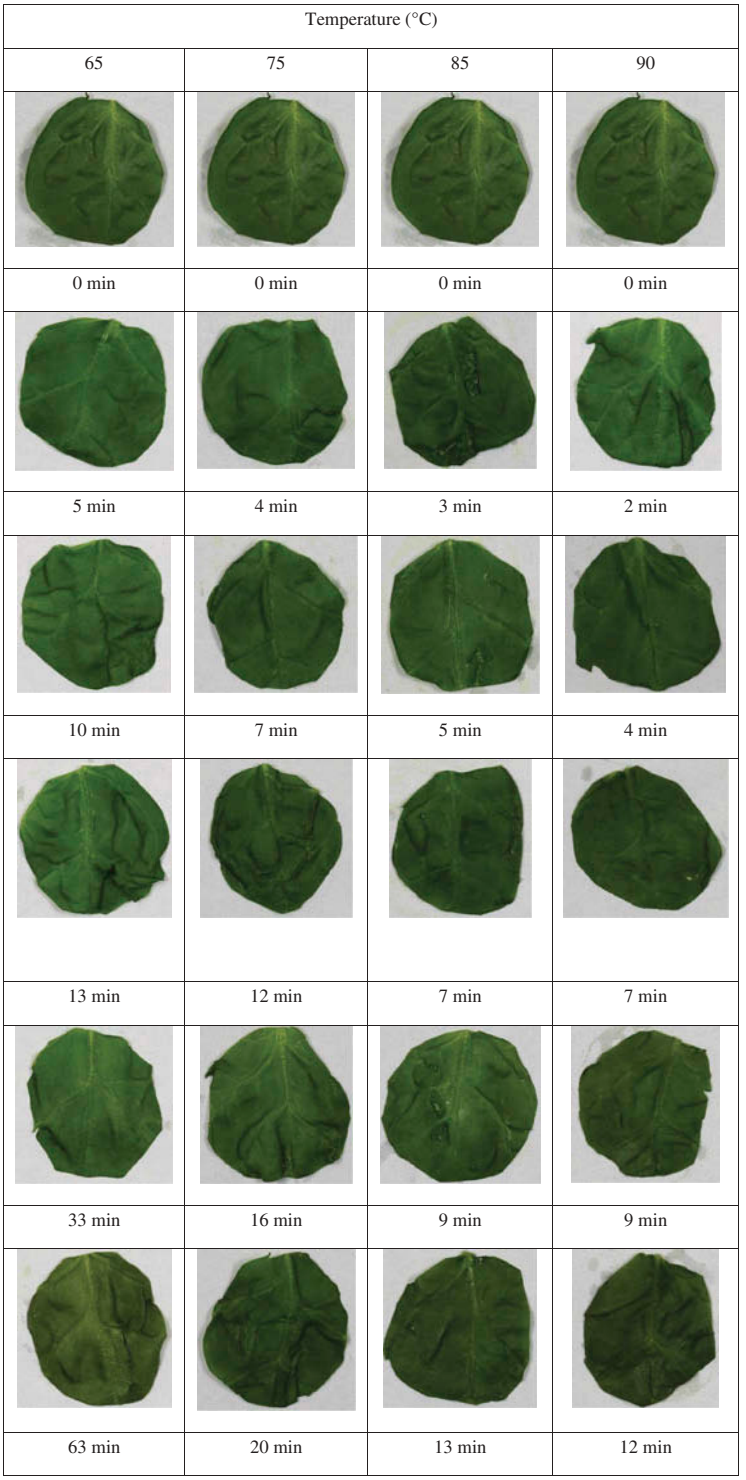

Figure 1 Visual color change in whole spinach leaves during different pasteurization treatments.

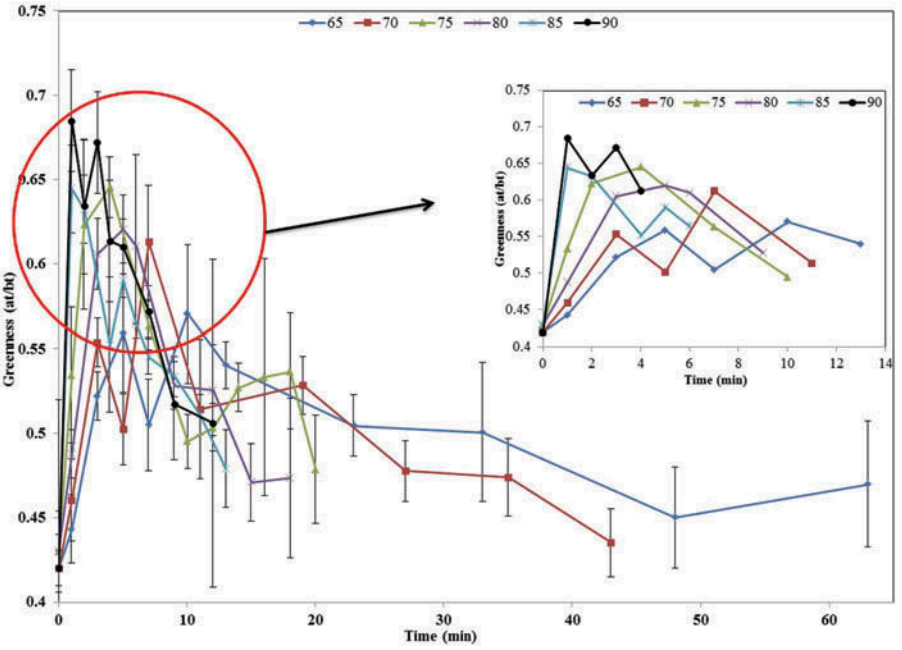

Figure 2 Changes in greenness during heat treatment of whole spinach leaves at different temperatures ($N = 3$) in winter.

The overall effect was that the green color increased during the initial stages of a short blanching process. With greater application of heat, chlorophylls were converted to pheophytins. During this process, hydrogen ions are substituted with Mg^{2+} in prophyrin ring in chlorophyll, which causes the formation of pheophytins. Chlorophyllase can act at moderate temperatures (65 to 75°C) hydrolyzing the phytol chain of pheophytins giving rise to pheophorbides, which decreased green color.[22,35]

The observed decrease in color later in the blanching treatment was most likely due to chemical degradation of chlorophyll[15] and a loss of the liberated colored compounds into extracellular water[9] decreasing color intensity. It was noted by Schwartz and Von Elbe[19] that pheophytin is only an intermediate in the thermal degradation of chlorophyll to pyropheophytin, a decarboxymethoxylated magnesium-free chlorophyll derivative. During the heating, the central magnesium atom of the chlorophyll porphyrin ring is easily removed, thus forming pheophytin. Upon prolonged heating, pheophytin degrades further, by decarboxymethoxylation of the isocyclic ring C-10 center, thereby forming pyropheophytin derivatives, which are the final degradation products of chlorophyll.[15,19] Colorimeter parameters (*L, a, b*) are associated with chlorophyll content.[36]

Seasonality has an effect on peak time and amplitude of maximum greenness, for example, in summer the peak time was 10 min for 65°C and 5 min for 85°C, while for autumn and spring it was 13 min for 65°C and 4 min for 90°C. Greenness was lower in summer and similar for other seasons. The seasonal differences are due to a higher concentration of chlorophyllase in spinach leaves during the summer as observed in July to September (summer) in green vegetables.[37]

The Effect of Temperature on the Visual Color of Spinach

Models predicting the loss of color during pasteurization treatments of whole spinach leaves are presented in Table 2. *L* values tended to be lower at lower treatment temperatures (65 and 70°C) and higher at higher treatment temperatures (75, 80, 85, and 90°C). In a study with spinach puree, Nisha et al.[12] found that the decrease in *L* value with continued heating was less at lower temperatures (50–70°C) than at higher treatment temperatures (80 and 120°C) but assumed that this difference in *L* value was not significant to food quality. In studies with microwave dehydration of spinach leaves, at higher microwave power, *L* value decreased at a lower time rather than at lower microwave power (3 min in 900 W, 18 min in 180 W).[2] At lower temperatures, chlorophyllase was activated during the thermal process, which reduced *L*, but at higher temperatures, the enzyme was inactivated resulting in greater brightness (*L*).[22]

Changes to Hunter *b* values tracked changes observed for measurement of greenness with a change in *L* and *b* value may be due to the pheophytin-pyripheophytin conversion

Table 2 Kinetic parameters at different temperatures as zero-order, first-order, and second-order models for *a, b*, and ΔE for heated spinach leaves.

	Peak time (min)				k (min^{-1})				E_a (KJ/mol)			
T (°C)[1]	SU[2]	AU	WI	SP	SU	AU	WI	SP	SU	AU	WI	SP
L												
65	10	13	10	13	0.00001	0.067	0.035	0.06	84.7	85.5	80	77
70	7	11	7	11	0.00003	0.0167	0.13	0.087				
75	7	10	4	7	0.0002	0.0162	0.07	0.1245				
80	6	6	5	6	0.000005	0.03	0.243	0.25				
85	5	5	1	5	0.0002	0.212	0.301	0.3				
90	—	4	1	4	—	0.41	0.26	0.36				
a												
65	10	13	13	13	0.0045	0.043	0.04	0.005	117.7	67.3	90.22	144
70	7	11	11	11	0.0014	0.11	0.133	0.017				
75	7	10	10	7	0.0146	0.23	0.3	0.09				
80	6	6	6	6	0.0122	0.214	0.15	0.14				
85	5	5	5	5	0.0278	0.3	0.31	0.19				
90	—	4	4	4	—	0.25	0.4	0.16				
b												
65	10	13	13	13	0.0291	0.0006	0.0011	0.0004	13.2	102.5	53.6	106
70	7	11	11	11	0.0144	0.0033	0.0061	0.0004				
75	7	10	10	7	0.1022	0.0028	0.003	0.002				
80	6	6	6	6	0.0276	0.0024	0.01	0.0033				
85	5	5	5	5	0.0288	0.0097	0.005	0.0009				
90	—	4	4	4	—	0.0114	0.04	0.0085				
ΔE												
65	10	13	13	13	0.0174	0.053	0.0405	0.0107	199.1	28.41	103.3	81.1
70	7	11	11	11	0.0659	0.05	0.33	0.017				
75	7	10	10	7	0.3329	0.254	0.12	0.066				
80	6	6	6	6	0.3818	0.053	0.4	0.284				
85	5	5	5	5	1	0.04	0.45	0.077				
90	—	4	4	4	—	0.22		0.085				

[1]Temperature (°C) range; 65 to 85°C for summer, 65 to 90°C for autumn, winter, and spring.
[2]SU: Summer; AU: Autumn; WI: Winter; SP: Spring.

or due to the degradation of other components present in spinach leaves.[15] Nisha et al.[7] noticed that for tomato puree, there is no constant change for *b* value under heat treatments and found a similar trend in a study with spinach.[10] Changes in Hunter *a* values are also reflected in changes in greenness. At all treatment temperatures, *a* value increased with increasing time. ΔE for pasteurized spinach leaves did not significantly change at 65°C but decreased at 70 to 85°C with the rate of decrease being higher at higher treatment temperatures. At 90°C, ΔE increased in autumn, winter, and spring reflecting lower levels of chlorphyllase compared to summer spinach.

Kinetic Models for Color Changes

The color parameters of fresh spinach were *L*: 43.9 ± 0.7 (summer), 39.2 ± 0.3 (autumn), 35.9 ± 1.0 (winter), 39.4 ± 0.6 (spring); *a*: -10.4 ± 0.5 (summer), -9.3 ± 0.3 (autumn), -8.6 ± 0.2 (winter), -9.0 ± 0.2 (spring); and *b*: 25.1 ± 1.8 (summer), 21.2 ± 0.8 (autumn), 20.2 ± 0.6 (winter), 20.7 ± 0.2 (spring). Mathematical models of color change for spinach leaves indicate that *a* was zero-order for all seasons, and *b* was zero-order for summer and first-order for the rest of the year, while ΔE was zero-order for summer, autumn, and winter, but first-order for spring. *L* value followed no apparent trend for summer and was zero-order for other seasons (Table 2). The peak time (Fig. 2) was selected as the first point of calculation. The kinetic rate constant increased with increasing temperature and is likely a reflection of greater heat transfer to the inside of the spinach leaves. Our findings for *a* and ΔE agree with studies for peach puree,[9,16] kiwi fruits,[38] and spinach during dehydration.[2] In some studies, first-order models have been found to more closely match the kinetic data for *a, b, L*, and total color change, for example, for spinach and mustard leaves purees,[18] green asparagus,[23] and spinach puree.[12] Differences in these findings may be due to different sample preparation methods, heating regimes, heating time, and heating sources. In a current study, a lower temperature range was studied, in which temperature dependent oxidation reactions observed at higher temperatures might not have occurred.

The activation energy (for *a, b, L*, and ΔE) was between 13.2 to 199.1 kJ mol^{-1}. Wide variations of activation of energy are reported by previous studies on green vegetable purees with limited data on whole tissue. Activation energies of 28.55 (spinach puree), 41.15 (mustard leaves puree), and 34.01 KJ mol^{-1} (a mixed puree of the mustard, spinach, and fenugreek), have been reported.[8] These variations may be attributed to the chemical, seasonal, and morphological differences in the plant tissues studied, and reflect differences between both whole tissue and purees. In a current study, the activation energy was different at different seasons, which could be explained by seasonal differences in the chemical composition of spinach leaves. The highest activation energy for ΔE was related to summer samples, which show the higher energy demand for changing the color in summer time. It might also have been related to the higher chlorophyll content in spinach leaves during the summer. A higher activation energy indicates a retarded rate of degradation and greater color retention as observed by Ahmed et al.[18] in a study of green chili puree.

Effect of Heat Treatments on Spinach Cell Morphology

The heat-treated samples clearly showed shrinkage and collapse of cells (Figs. 3b–3d, 3f) when compared to untreated samples (Figs. 3a, 3e). The shrinkage of cells and structural changes may have influenced the color readings. Dadali et al.[21] reported that

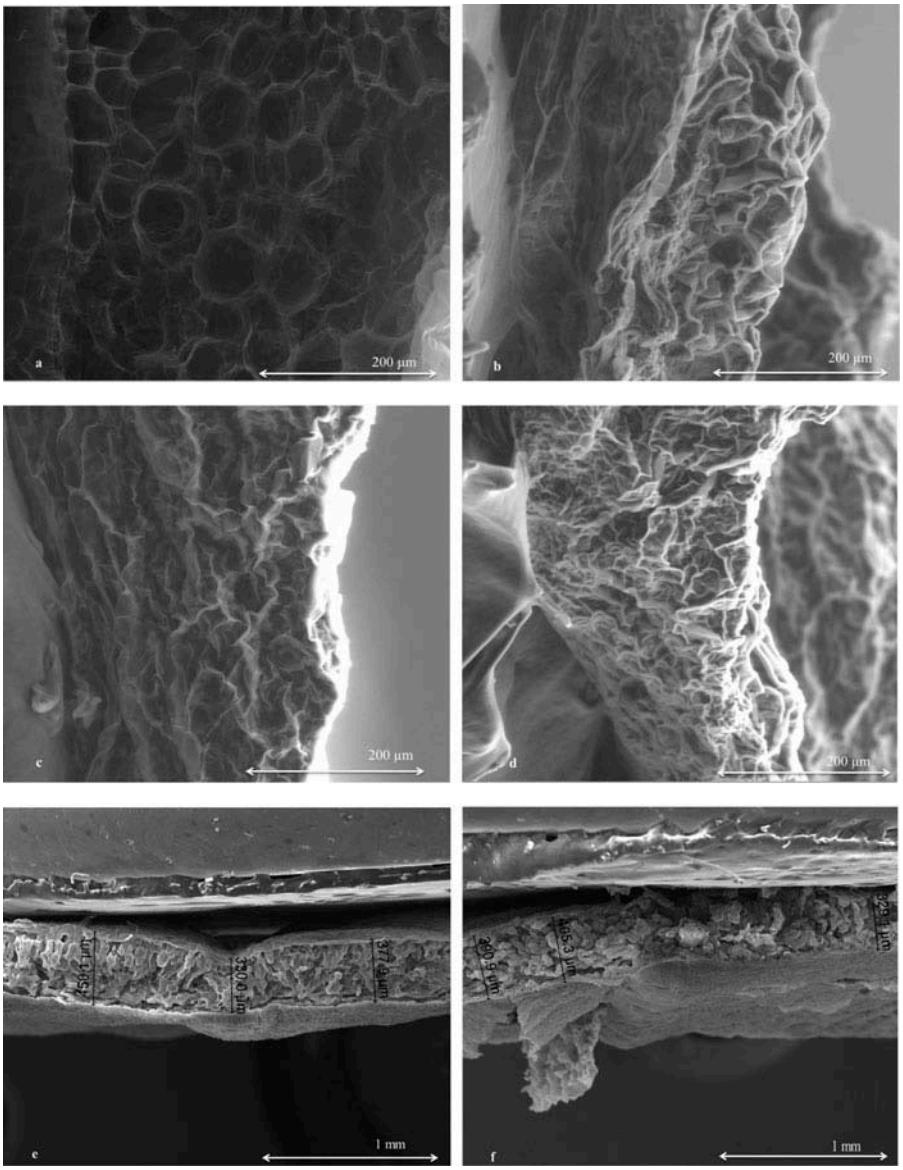

Figure 3 SEM of non-heat-treated and heat-treated spinach leaves: (a) Control, (b) 65°C for 63 min, (c) 75°C for 20 min, (d) 85°C for 13 min, (e) Control leaf cross section, and (f) 85°C for 13 min leaf cross section.

the cell shape changed when increasing microwave power during microwave dehydration of spinach leaves. Microwave drying can also increase porosity.[39] Shrinkage could be calculated as a change in cross section, for example, between control (e) (386 ± 60 µm) and heat treated (f) 85°C for 13 min) (375 ± 40 µm) approximately a 3% decrease.

CONCLUSION

Greenness (a_t/b_t) increased during the initial stages of pasteurization at all treatment temperatures and for spinach from different seasons, but the magnitude of the increase was greater at 75–90°C than at 65 and 70°C and was lowest for spinach harvested in the summer. The peak time was longer and amplitude lower for summer spinach compared to samples collected during the rest of the year because of higher enzyme (chlorophyllase) activity. Mathematical models of color change for spinach leaves indicated seasonal differences in chemical composition resulting in different reaction orders. The activation energies reported here are higher than those of other researchers and may be due to the lower temperature range tested. Different mechanisms for loss of greenness may be at play, with enzymatic activity playing a prominent role at lower temperatures and cell collapse, cell compaction, and oxidative changes being more important at higher temperatures. In addition, greenness exhibited seasonal differences. In general, the natural color of whole spinach leaves can be retained to a greater extent at a lower temperature. The kinetic equations developed can be used for optimizing pasteurization processes to maximize color retention.

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