

Temperature abuse timing affects the rate of quality deterioration of commercially packaged ready-to-eat baby spinach. Part I: Sensory analysis and selected quality attributes



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ABSTRACT

Temperature abuse of fresh-cut products occurs routinely during transport and retail store display. However, the stage of product shelf life during temperature abuse and its impact on sensory attributes have not been studied. This study evaluated the effect of temperature abuse occurring immediately after processing and late in shelf life through measurements of sensory attributes, and membrane integrity of commercially packaged ready-to-eat baby spinach. The packaged products were received within 2 days of processing. Samples subject to early temperature abuse were immediately placed at 1, 4, 8, 12, 16 and 20 °C storage upon arrival, and those subject to late temperature abuse were stored at 1 °C for six days, and then transferred to 4, 8, 12, 16 and 20 °C storage. Package headspace gas composition, in-package visual appeal, purchase intent, product color, off-odor, decay, texture, overall quality, and tissue electrolyte leakage were evaluated every 1–2 day up to 16 day total. Results indicate that when the product temperature is maintained at 1–4 °C, the quality of commercially packaged baby spinach can be retained for up to 18 days post-processing. However, storage temperature of 8 °C or above, significantly ($P < 0.001$) shortened product shelf life as exhibited by accelerated tissue electrolyte leakage, product yellowing, decay and off-odor development. Most importantly, the product's shelf life stage significantly affected its response to temperature. Quality deterioration proceeded more rapidly when temperature abuse occurred in late as opposed to early shelf life stage.

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1. Introduction

Consumers have become more health conscious and actively demand ready-to-eat, nutritious packaged minimally processed vegetables. However, tissue injuries sustained during fresh-cut processing promote faster physiological deterioration, induces undesirable biochemical changes in the product, and facilitate microbial proliferation. Spinach is an annual cool season crop and commonly grows in temperate areas. Baby spinach leaves contain relatively high levels of bioactive compounds such as vitamin C, vitamin A and minerals (Pandrangi and Laborde, 2004). Similar to other fresh-cut vegetables, although not cut, the washing,

drying/dewatering, and packaging steps involved in the preparation of packaged “ready-to-eat” baby spinach stimulate tissue deterioration. Major defects reported for packaged baby spinach leaves are the development of unpleasant odors, decay, discoloration, and loss of turgor (Medina et al., 2012; Tudela et al., 2013; Watada and Qi, 1999; Wang, 2003).

Temperature management is often the most effective way to delay deterioration and maintain the quality of fresh-cut fruits and vegetables (Jacxsens et al., 2002; Luo et al., 2009). Many studies have shown that lowering the storage temperature can significantly reduce the growth of spoilage microorganisms and delay the physiological deterioration of plant tissues (Jacxsens et al., 2002; Luo et al., 2009; Smyth et al., 1998). Enriquez et al. (2000) observed that lower temperatures were effective in decreasing Malabar spinach yellowing, ascorbic acid loss and respiration rate. Pandrangi and Laborde (2004) reported that spinach color and appearance became unacceptable after 8, 6, and 4 d at 4, 10, and 20 °C, respectively. Luo

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et al. (2009) observed that product quality scores for commercially packaged baby spinach held at 12 °C remained high for the first 6 d of storage and then declined sharply by d 9. Processors recommend that fresh-cut vegetables be stored at 1–3 °C to maintain product quality for maximal shelf life.

Low temperature should theoretically be maintained throughout product distribution, retail and home use (Kader, 2002; Willocx et al., 1994). However, the distribution chain rarely has the facilities to store each commodity under optimal conditions and requires compromises about choices for storage temperature. In reality, sub-optimal temperature exposure occurs frequently during fresh-cut product distribution and in retail display cases (Koseki and Isobe, 2005). In the United States, 20% of domestic and commercial refrigerators operate at a temperature of >10 °C (50 °F) (Jol et al., 2006). A survey of processed vegetables in Belgian retail display cabinets also showed that there are more than 5 °C temperature differences in the decks of retail display cabinets (Willocx et al., 1994). In France, 50% of the domestic and 32% of the commercial refrigerators had temperatures greater than or equal to 9 °C (Taoukis et al., 2005).

Traditionally, “first-in-first-out” for intact fruits and vegetables, and “best if used by date” have been used for inventory management of fresh and fresh-cut produce. Increased understanding of the important impact of temperature abuse on product quality deterioration has led to the development and application of time and temperature based inventory management software programs, and end user oriented time temperature indicators/integrators (TTI) for fresh and fresh-cut produce. However, almost all of these technologies available today were developed based on a model which tracks the time lapse for which storage temperature exceeds the threshold temperature and overlook the confounding factor of produce shelf life stage on their response to temperature abuse (Bobelyn et al., 2006; Taoukis et al., 1999). Although no specific scientific information are available documenting such impact, a number of studies have shown that a product's shelf life stage or physiological condition significantly affects its response to environmental stressors (Arora et al., 2002; Zhou et al., 2004; Pandrangi and Laborde, 2004; Francis and O'Beirne, 2001). Therefore, the objective of this study was to evaluate the effects of temperature variations occurring at different stages of product storage life on the changes in sensory quality of packaged baby spinach over time. Data obtained will provide pertinent information to the industry on how to factor shelf life stage into the development of inventory management systems and thus further improve the accuracy and usefulness of these systems for perishable fresh and fresh-cut produce.

2. Materials and methods

2.1. Sample and storage facility

Commercially packaged baby spinach (70 cases = 420 bags) was obtained from Dole Fresh Vegetables, Inc. (Bessemer City, North Carolina, USA). Spinach leaves were machine harvested, cooled to below 5 °C within 4 h, triple washed, and packaged in laser-perforated bags. Biological variation between packages of baby spinach was minimized by obtaining product processed from the same line and shift. The samples were transported by a commercial refrigerated truck (2–4 °C) to the Food Quality Laboratory (Beltsville, MD), and were immediately sorted and labeled at 1 °C and then placed at their assigned temperature storage. Prior to arrival of the samples, cold rooms were equilibrated at the assigned temperatures. Data loggers were used to record the temperature in each room every 5 min during the 16 d storage period (Log Tag temperature recorder, TRIX-8, MicroDAQ.com, Ltd., Contoocook, NH, USA).

2.2. Temperature abuse 2 days (early stage) and 8 days (late stage) post-processing

In order to simulate temperature abuse occurring at early stage post-processing, on arrival day (d 0), five replicate bags of packaged baby spinach were placed in cold storage rooms with temperatures maintained at 1, 4, 8, 12, 16 and 20 °C. The samples were evaluated on d 0, 1, 2, 4, 6, 8, 10, 12, 14 and 16. Late stage temperature abuse was modeled by storing bags at 1 °C for 6 d, and then transferring them to 4, 8, 12, 16 and 20 °C storage. The evaluations were performed for these samples on d 6, 7, 8, 9, 10, 12, 14 and 16. The evaluation was discontinued when either scores for purchase intent or visual quality declined to 40 or below (out of 100), or off-odor reached or exceeded 60 (out of 100), or the overall quality fell below 50 (out of 100), as these values indicated that the product was no longer marketable (López-Gálvez et al., 1996; Zhou et al., 2004).

2.3. Analysis of packaging headspace composition

The package atmospheres were measured immediately upon removal of the samples from storage. The O₂ and CO₂ concentrations of the samples were analyzed using a gas analyzer (Check mate II, PBI Dansensor Co., Denmark) by inserting the needle of the measuring assembly through a septum adhered to the packaging film.

2.4. Sensory attributes

Sensory attributes of baby spinach leaves were evaluated at the Food Quality Laboratory Sensory Facility consisting of ten individually partitioned booths equipped with individual computers. The products were evaluated with a trained five member sensory panel. Prior to the evaluation, training sessions were provided for the panelists on scoring the quality attributes. For decay scores, the decayed samples from preliminary studies were grouped by the panelists based on severity and extent of decay. The samples were photographed and the sample images and the scores were used for additional panel training. All quality attributes were rated using unstructured 100-mm scales. On-screen ballots were prepared and data were collected using Compusense Five program (Version 5.4) (Compusense Inc., Guelph, Canada). The sealed sample bags were coded with random 3-digit numbers and presented to the panelist for the evaluation of ‘visual appeal’ and ‘purchase intent’. Visual appeal was rated from poor (0) to excellent (100) based on the product appearance viewed from the non-printed areas of the package film. Purchasing intent was rated from definitely would not buy (0) to definitely would buy (100). Baby spinach leaves were then transferred to sampling trays (20–25 g per tray) and panelists evaluated the off odor, decay extent, texture, and overall quality. The off-odor was determined by smelling the product on the tray, and assigned a rating from none (0) to very strong (100). Decay was examined based on the severity and prevalence of decayed leaves according to a pre-determined scale; none (0) to severe (100). Texture was evaluated by folding and breaking over 10% of the leaves using fingers and rated from flaccid (0) to very crisp (100). Overall quality was rated from poor (0) to excellent (100), following a similar procedure by Saftner et al. (2002). The acceptable or marketable range for visual quality, purchase intent and overall quality was considered to be a score of 60 or above, while an off-odor and decay score of 40 or below was considered to be acceptable.

2.5. Color assessment

Each sample was composed of approximately 35 g of baby spinach leaves that were placed on a white tray (17 cm × 13.5 cm × 3 cm) and color coordinates (L*, a*, b*) were

measured directly on the baby spinach leave using a colorimeter (Konica Minolta CR-410 Chroma Meter, Ramsey, NJ) with a 50 mm diameter viewing aperture. The instrument was calibrated with a white tile ($Y=94.0$, $x=0.3130$ and $y=0.3191$). Color was measured at 10 random locations on each sample. The a^* and b^* color coordinates were converted into hue angles [$\text{hue} = \tan^{-1}(b/a)$], where 0° = red purple, 90° = yellow, 180° = bluish green, and 270° = blue.

2.6. Tissue electrolyte leakage

Tissue electrolyte leakage was measured following a modified procedure from [Luo et al. \(2009\)](#). Fifteen grams of spinach leaves were removed from each package and submerged in 300 mL of deionized water for 30 min at 20°C . The electrolyte content of the solution was determined by measuring the electrical conductivity with a conductivity meter (model 135A, Orion Research, Inc., Beverly, MA). Total electrolytes of the baby spinach samples were determined after freezing at -20°C for 24 h and thawing at room temperature. Tissue electrolyte leakage was expressed as a percentage of total electrolytes.

2.7. Experimental design and statistical analysis

Five replications (five bags) per treatment per evaluation period were examined. Package atmospheres, tissue electrolyte leakage, color and sensory data were analyzed as a two-factor (storage temperature and duration) linear model using the PROC MIXED procedure (SAS Institute Inc., 1999, Cary, NC). Assumptions of normality and variance homogeneity of the mixed model were checked

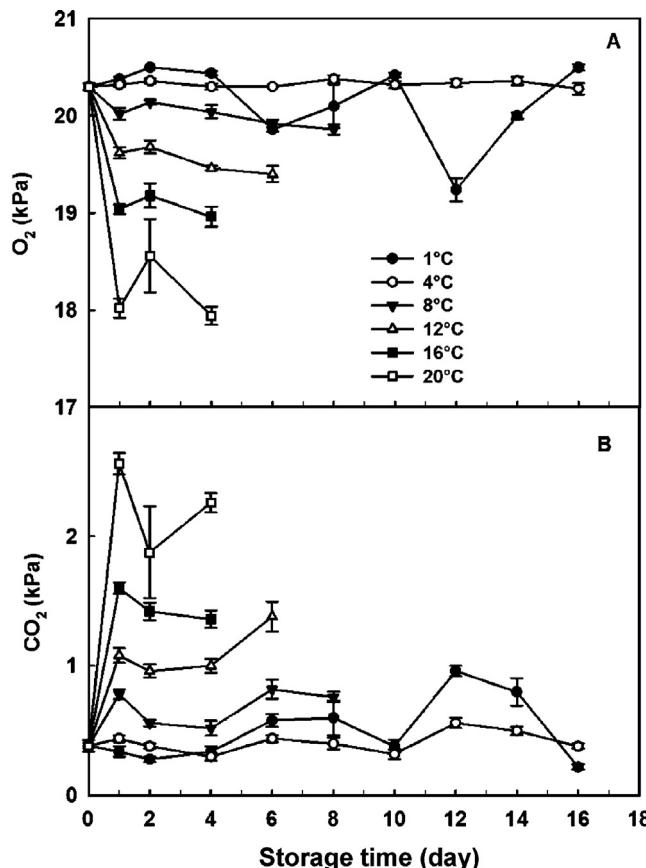


Fig. 1. Effect of early temperature abuse initiated 2 d after processing on O_2 (A) and CO_2 (B) of baby spinach stored at a range from 1 to 20°C following 0–16 d storage. Data presented are the means of five replications; vertical lines represent standard errors.

and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means were compared using Sidak adjusted p -values to maintain experiment-wise error ≤ 0.05 . For sensory data, treatment differences were tested with Tukey-Kramer test, $\alpha = 0.05$.

3. Results and discussion

3.1. Package headspace composition

Temperature management is often the single most important option to delay the deterioration of fresh-cut fruits and vegetables while simultaneously maintaining their quality ([Jacxsens et al., 2002](#); [Kader, 2002](#)). Storage temperature influences the respiration rate, metabolic processes, and hence, the senescence rate of produce ([Jacxsens et al., 2000](#)). Within a defined package configuration, headspace gas composition is an indication of the product's respiration rate, with lower oxygen and higher carbon dioxide levels indicating increased respiration. Although, baby spinach packages are micro perforated and thus have high gas exchange rates, differences were apparent between packages stored at different temperatures. [Figs. 1 and 2](#) show the headspace compositions of baby spinach packages stored at different temperature regimes. Overall reductions in O_2 and increases in CO_2 levels were observed in baby spinach over time. There was a rapid decrease in O_2 on

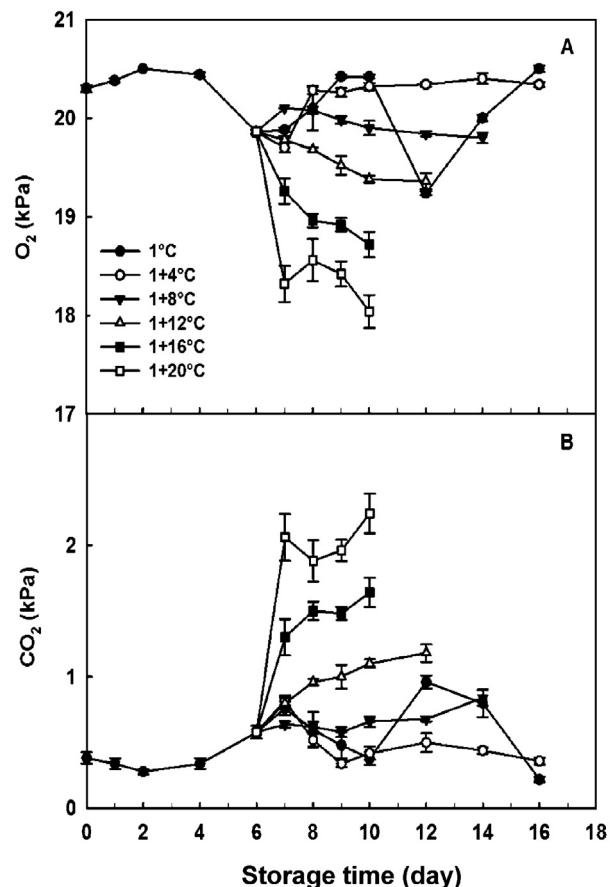


Fig. 2. Effect of late temperature abuse initiated 8 d after processing (d 6 of study) on O_2 (A) and CO_2 (B) of baby spinach stored at 1°C for 6 d and then transferred to storage at temperatures 4 – 20°C for the remainder of shelf life. Headspace gas measurements were taken from 0 to 10 d after transfer. Temperature regime treatment codes for baby spinach transferred to higher temperatures on d 6 are $1+4$, $1+8$, $1+12$, $1+16$ and $1+20^\circ\text{C}$. Data presented are the means of five replications; vertical lines represent standard errors.

the first day except in packages stored at 1 and 4 °C (Fig. 1A). After an initial equilibration period, the package atmospheres remained stable until the end of the storage period. Samples stored at 20 °C had the lowest O₂ and highest CO₂ values, followed by those stored at 16, 12, 8, 4 and 1 °C.

The changes in O₂ and CO₂ in the baby spinach packages stored at 1 °C for 6 d then transferred to different temperatures followed similar trends to those where different temperature regimes were

established early during the product shelf life. The exception was that packages exposed to temperature abuse that occurred in the earlier period of shelf life maintained a higher O₂ and lower CO₂ concentration in the headspace than those where temperature abuse occurred toward the end of shelf life (Fig. 2A and B).

The O₂ and CO₂ values measured for the packages in Figs. 1 and 2 were similar to those obtained in previous studies for baby spinach (Luo et al., 2009). Surprisingly, a slight reduction in O₂ and rise in

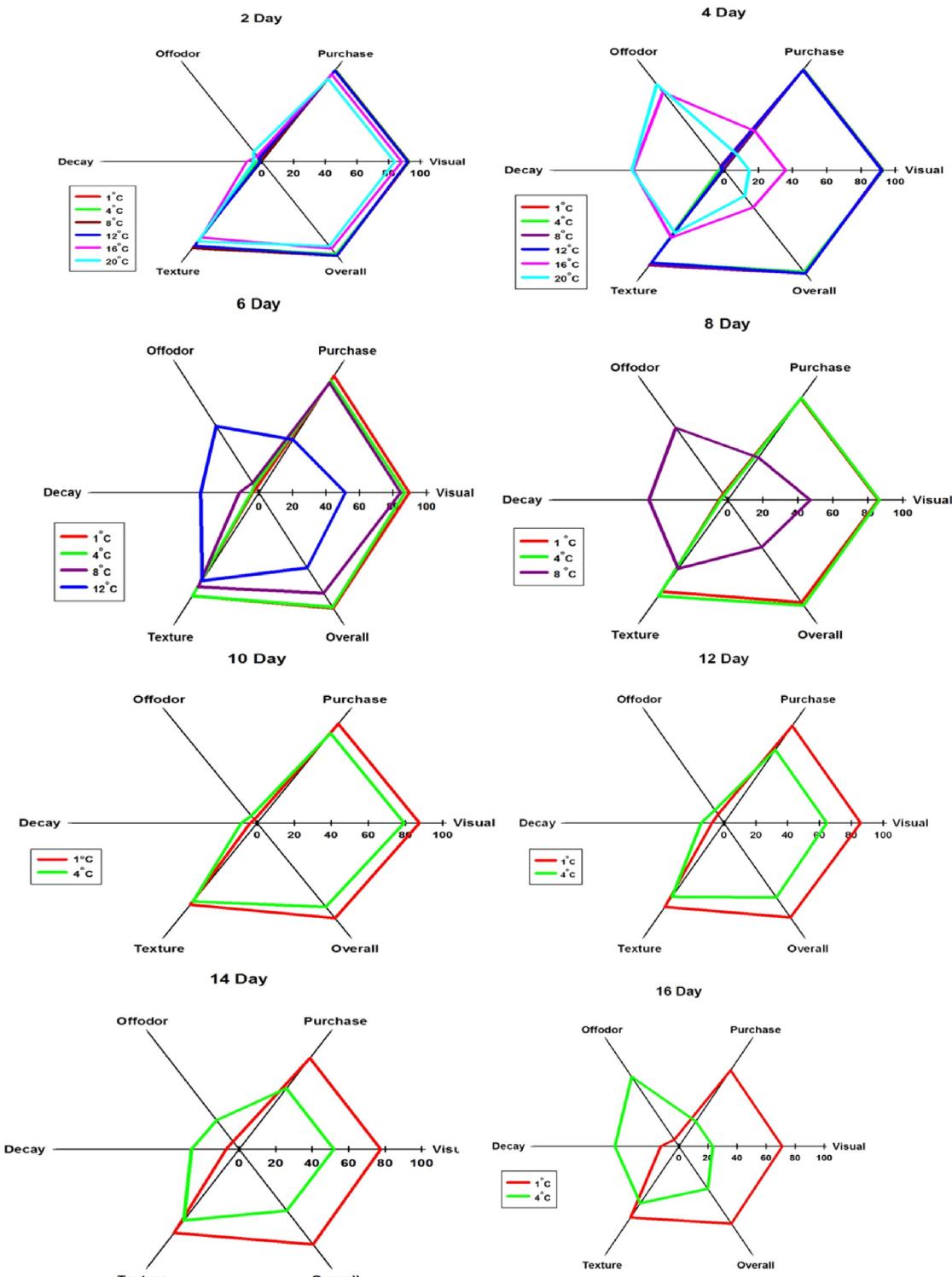


Fig. 3. Effect of abusive temperatures occurring early during shelf life on sensory attributes of baby spinach after 0 to 16 d. Storage temperatures initiated on d 0 of study are 1, 4, 8, 12, 16 and 20 °C. Data presented are the means of five replications; vertical lines represent standard errors.

CO_2 levels at 12 d were observed in samples stored at 1 °C, with O_2 levels exceeding and CO_2 levels lower than those of samples stored at 4 °C for both d 12 and 14. The exact reason for this sudden change in the trend for gas composition is unknown. However, the shift in spinach respiratory activities in these temperature ranges may have played a role. Pandrangi and Laborde (2004) also observed an unexpectedly rapid loss of folate and growth of mesophilic and

psychrotrophic bacteria in packaged spinach at 4 °C rather than at 10 °C.

3.2. Sensory analysis

Significant differences in the sensory attributes were observed among the baby spinach samples stored at different temperatures

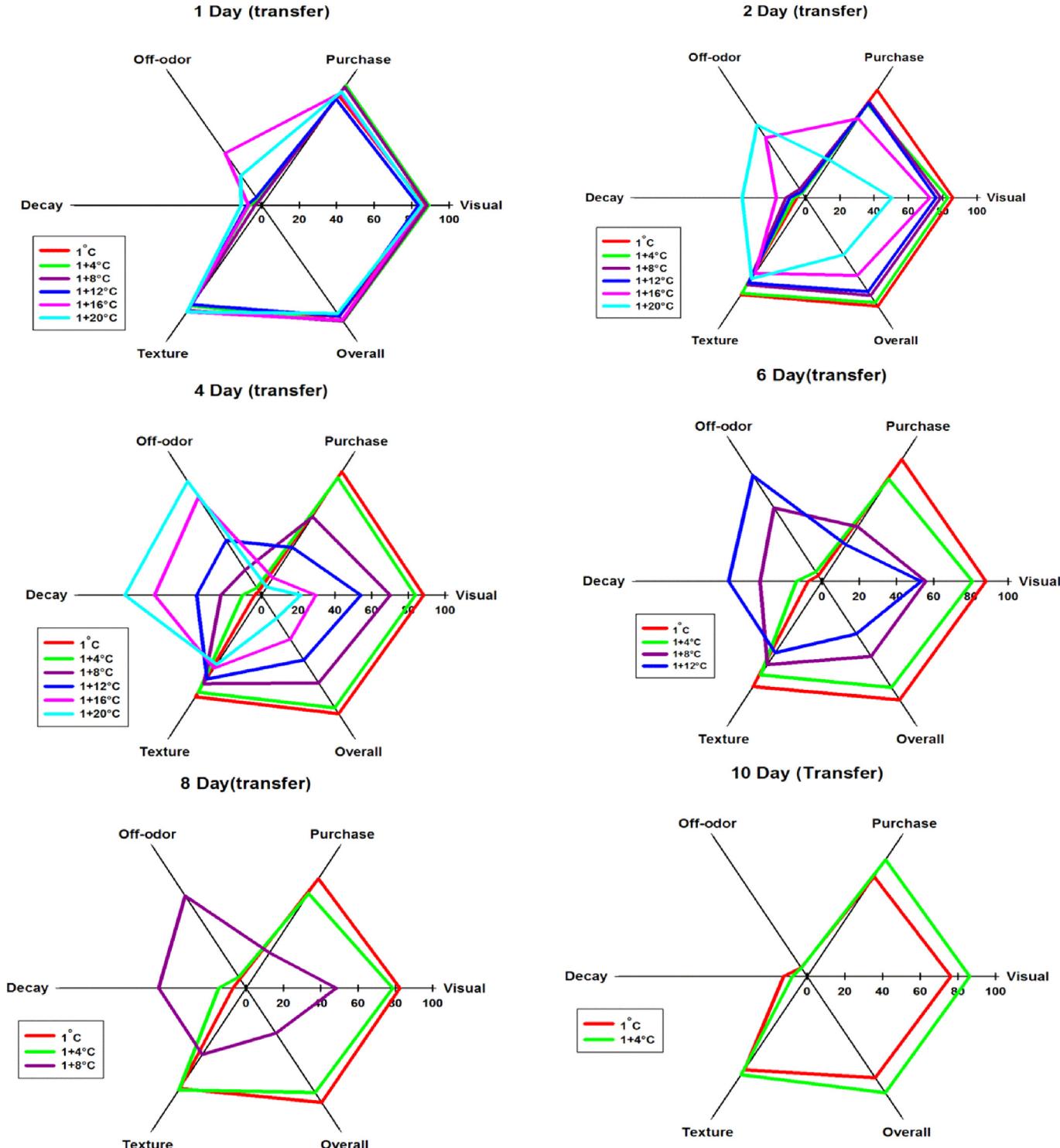


Fig. 4. Effect of abusive temperatures occurring late during shelf life on sensory attributes of baby spinach. After 6 days storage at 1 °C samples were transferred to 4, 8, 12, 16 and 20 °C. Sensory evaluations were conducted on 1 to 10 d after transfer. Temperature regime treatment codes for baby spinach transferred to higher temperatures on d 6 are 1+4, 1+8, 1+12, 1+16 and 1+20 °C. Data presented are the means of five replications; vertical lines represent standard errors.

($P < 0.001$) over time ($P < 0.001$) (Fig. 3). All sensory attributes scored high (near 100) for d 0 samples, indicating the pristine condition of the products received from the processor 2 d after packaging. One day after storage, samples at 20 °C showed signs of quality deterioration, while samples from all other treatments maintained the same sensory scores from d 0 (data not shown). On d 2, samples stored at 20 and 16 °C exhibited quality deterioration, while all other samples scored high in visual quality, purchase intent, overall quality and had no off-odor or decay. On d 4, samples stored at 20 °C had a sharp decline in visual quality and developed a strong off-odor, significant decay, and were thus removed from storage. Samples stored at 16 °C had better quality than those stored at 20 °C, but the overall quality fell below the acceptable level. No difference in sensory attributes were noted for samples stored at 1, 4, 8, and 12 °C as they all retained high quality. On d 6, samples stored at 12 °C had declined, the overall quality dropped to a score of 57.8. Samples stored at 8 °C also began to decline in quality, although were acceptable with overall quality score of 77.3. On d 8, samples stored at 8 °C showed a rapid decline in quality compared to d 6 as off-odor, decay, and overall quality scores fell below the acceptable range. No difference was noted for samples stored at 1 and 4 °C and both were of high quality. On d 10, samples stored at 1 and 4 °C started to show differences in visual quality, purchase intent, and overall quality, while texture remained good, and off-odor was minimal. From d 10 to d 14, differences between 1 and 4 °C storage were evident, but both treatments remained in acceptable the range. On d 16, samples stored at 4 °C had overall quality that fell below the acceptable range while those stored at 1 °C were fully acceptable, with all positive quality attributes rated at 70 (out of 100) and minimal off-odor and decay (6.3 and 12.5, respectively).

The changes in sensory attributes for baby spinach stored at different temperatures toward the end of shelf life followed the same trend as those samples placed in different temperature regimes in the earlier stages of shelf life; but the quality deterioration in the later group proceeded at a significantly faster pace (Fig. 4). Within 1 d after storage at 20 °C, baby spinach samples developed significant off-odor and signs of decay (data not shown). On d 2, samples stored at 16 and 20 °C had a significant decline regarding visual quality, purchase intent, and overall quality; and off-odor and decay were evident. Samples stored at 8 and 12 °C also significantly declined in quality. From d 2 to d 4, there was a drastic increase in off-odor and decay and a concomitant decline in visual and overall quality for samples stored at 20 and 16 °C that were below the acceptable range. Samples stored at 8 and 12 °C showed a further decline in quality although remained in the acceptable range. On d 6, a significant decline in quality and development of off-odor and decay was noted in samples stored at 12 °C and they were removed from the study. On d 8, samples stored at 8 °C were unacceptable.

In comparison between early and late shelf life temperature abuse, a significantly faster quality deterioration was noted when the abuse occurring at a later point in the products shelf life (comparing Figs. 3 and 4). Although, samples stored at 16 and 20 °C reached the end of their shelf life after 4 d, there was a much stronger off-odor and decay and a lower visual and quality score for those samples receiving temperature abuse in later stages. During the early stage of temperature abuse, samples stored at 8 and 12 °C maintained high quality after 4 d, yet those receiving late stage temperature abuses had noticeable quality deterioration occurring within 2 d after initiation of storage at these temperatures and there was substantial decline in quality after 4 d storage at 8 and 12 °C. Interestingly, although samples stored at 4 °C early in storage received lower quality scores than those stored at 1 °C, at the end of the storage regime when there was no temperature shift as expected (Fig. 3), better quality was observed on samples stored at 1 °C for 6 d, followed by 4 °C for the remainder of storage than on those stored at 1 °C for the entire storage time (Fig. 4).

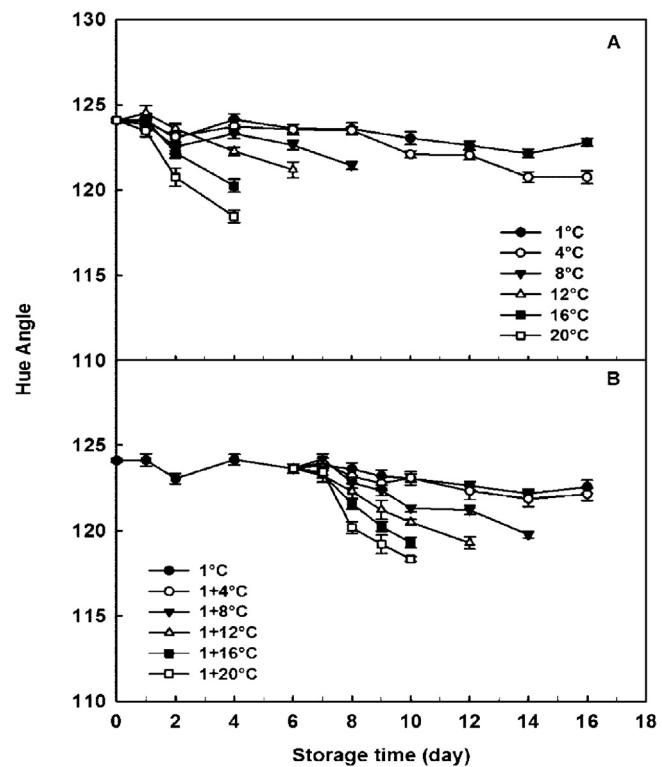


Fig. 5. Effect of abusive temperatures occurring early during shelf life (A) and late in shelf life (B) on hue angle of baby spinach after 0–16 d. Storage temperatures initiated on d 0 of study are 1, 4, 8, 12, 16 and 20 °C. Temperature regime treatment codes for baby spinach transferred to higher.

3.3. Color

The temperature effect on the changes in color followed the same trend as that observed for sensory attributes, although less pronounced. Decreases in hue angles were observed on samples stored at 20, 16, 12, and 8 °C, with higher temperatures resulting in faster decline in hue angle. Similar temperature effect was found with the increase in b^* values (data not shown). These changes coincide with the gradual shifting of leaf color from dark green to yellow. Early and late stage temperature abuse exhibited similar trends, but late stage temperature abuse had a more pronounced detrimental effect (comparing Fig. 5A and B). The progressive changes in color at higher storage temperature suggest a gradual loss in chlorophyll during storage (Fang et al., 1998; Hodges et al., 2000). Ludford (2003) also reported similar leaf yellowing and rapid chlorophyll loss caused by high storage temperatures.

3.4. Tissue electrolyte leakage

Electrolyte leakage has frequently been used as an indicator for tissue and membrane integrity in postharvest quality analyses, and previous studies have shown its close correlation with product quality and shelf life (Kou et al., 2012; Luo et al., 2004). Storage temperature and duration significantly affected electrolyte leakage of baby spinach samples (Fig. 6A). Samples stored at 1 °C had relatively low electrolyte leakage until after 12 d in storage whereas a significant increase was observed in all other samples. The general trend was that the higher the storage temperature, the more rapid increase in electrolyte leakage. After 4 d in storage, electrolyte leakage increased 11-, 4- and 4-fold for samples stored at 20, 16 and 12 °C, respectively, compared with the initial values or those of samples stored at 1 °C. After 14 d of storage, samples at 4 °C had significantly ($P < 0.01$) higher electrolyte leakage than those at 1 °C,

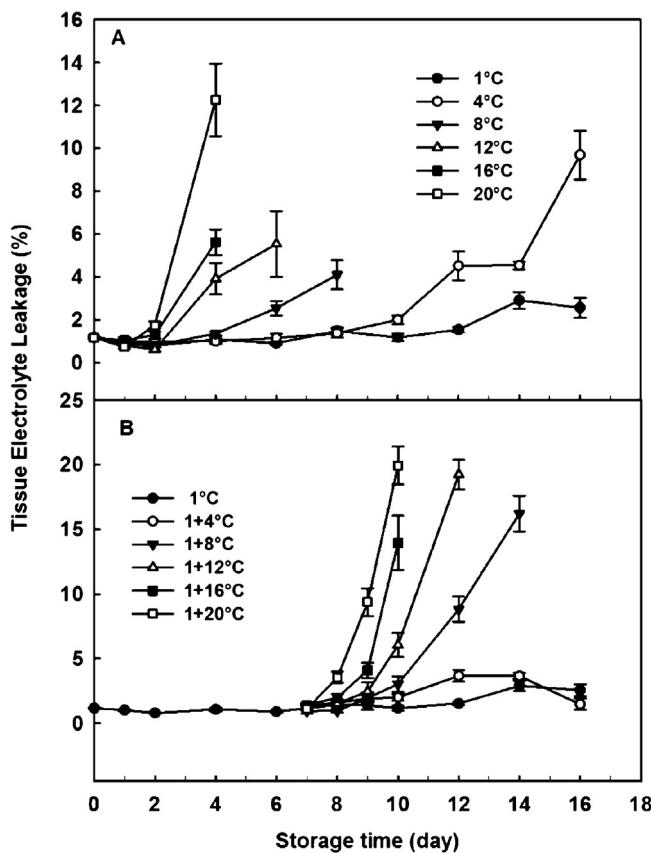


Fig. 6. Effect of abusive temperatures occurring early during shelf life (A) and late in shelf life (B) on tissue electrolyte leakage of baby spinach after 0–16 d. Storage temperatures initiated on d 0 of study are 1, 4, 8, 12, 16 and 20°C. Temperature regime treatment codes for baby spinach transferred to higher temperatures on d 6 are 1+4, 1+8, 1+12, 1+16 and 1+20°C. Data presented are the means of five replications; vertical lines represent standard errors.

reaching 9.7%. These results are in agreement with previous reports (Allende et al., 2004; Luo et al., 2009).

Similar trends were observed for electrolyte leakage of baby spinach samples stored at abusive temperatures that occurred at the beginning and end of the product's shelf life. However, electrolyte leakage occurred more quickly in samples when temperature abuse occurred at the end of the product shelf life, than when temperature abuse occurred early which coincided with quality deterioration. As shown in Fig. 6B, tissue electrolyte leakage of samples increased 20-, 14- and 6-fold on d 10 for 1+20, 1+16 and 1+12°C, respectively, compared with those samples stored at 1°C. The observed acceleration of the detrimental effect of temperature abuse occurring at late shelf life stage may be associated with the physiological condition of the products. In the late stages of product shelf life (after 6 d of storage), the product has already partially senesced, stored carbohydrates have been consumed, cell wall disassembly has progressed, thus the leaves become fully senesced more rapidly once exposed to elevated temperatures. Since temperature abuse incidents often occur during the retail store displays when products reached their later shelf life stage, the findings observed in this study underscore the importance of maintaining cold chain integrity throughout the entire supply chain. Furthermore, when developing time temperature based inventory management systems, our findings demonstrate that it is critical to factor shelf life stage of potential temperature abuse or sub-optimal temperature storage occurrences.

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