



# Open-refrigerated retail display case temperature profile and its impact on product quality and microbiota of stored baby spinach



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## ABSTRACT

Maintaining proper storage temperature is critical for ensuring the quality and safety of fresh-cut products. The US Food and Drug Administration Food Code recommend that packaged fresh-cut leafy green vegetables be kept no warmer than 5 °C at all times to ensure food safety. Substantial temperature variations, however, within the widely used open refrigerated display cases used in retail stores are known to present the technical challenge of complying with this federal guidance for industry. This study determined the extent of the spatial and temporal temperature variations within two commercial open-refrigerated display cases under different operating conditions, and their impact on the quality and microbial growth of packaged baby spinach products. The packaged products were received within 2 d of commercial processing and temperature data loggers were placed inside-and-outside of each bag. All bags were immediately loaded in the display cases and the overall visual quality, tissue electrolyte leakage, total aerobic mesophilic bacteria and psychrotrophic bacteria were evaluated for each bag. Results from this study showed that the temperature variation in the cases was dependent on spatial location, thermostat setting, and defrost cycle interval and duration of defrost. The largest temperature differentials were found for samples located in the front and back rows of the display cases. Samples located in the front rows had the highest temperature due to heat penetration from the surrounding ambient environment, while those in the back were damaged as temperatures fell below freezing. These products received low quality scores and had higher tissue electrolyte leakage. In order to reduce the large temperature variations in the display cases, insulating foam boards were installed which significantly ( $P < 0.05$ ) decreased the temperature variation by 3.5 °C and enabled samples in the front rows of the cases to remain less than 5 °C as recommended by the FDA. These results suggest that the quality and safety of packaged ready-to-eat spinach at retail will benefit from improvements in open refrigerated case design or the utilization of insulation, doors or curtains.

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## 1. Introduction

Packaged fresh-cut vegetables are popular food products as they are convenient, healthy, and ready-to-eat. Due to increased consumer demand, the fresh-cut produce industry has been rapidly expanding. However, fresh-cut produce has limited shelf stability due to rapid product quality deterioration. Advancements in fresh-

cut packaging technologies have enabled the industry to maintain quality for longer periods of time (approximately 14 d) (Chua, Goh, Saftner, & Bhagwat, 2008; Kim, Luo, Saftner, & Gross, 2005). The ability to maintain recommended storage temperatures of these fresh-cut products is vital to ensure optimal food quality, and extended product shelf life (Evans, Scarcelli, & Swain, 2007).

Storage temperature is an important factor affecting the growth of spoilage and pathogenic bacteria. Studies from Luo, He, McEvoy, and Conway (2009) and Luo, He, and McEvoy (2010) showed that *Escherichia coli* O157:H7 grows rapidly in temperature-dependent manner on bagged fresh-cut leafy green products. The US Food and Drug Administration (FDA, 2009) revised the Food Code to include packaged fresh-cut leafy greens in the “temperature control

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for safety” (TCS) food category, and recommended that these products be maintained at 5 °C or below in transport, storage and retail display. This has also been adopted in the 2013 version of the Food Code (FDA, 2013).

Open-refrigerated display cases are widely used in supermarkets and grocery stores as a primary means to provide the cold temperature necessary for the proper storage of fresh and fresh-cut produce. Although these cases have the visual benefit of being aesthetically pleasing with concomitant easy access by consumers, they are not energy efficient and often fail to provide the temperature necessary for proper storage of the packaged fresh-cut fruits and vegetables. Surveys conducted by Willocx, Hendrick, and Tobback (1994) showed that 30% of products presented in Belgian refrigerated display cases were subjected to temperature variations and abuse in commercial settings. Evans et al. (2007) further reported that the majority of high temperature abuse (97%) was located at the front rows of the open-refrigerated display cases and that refrigeration systems and designs of retail displays were not sufficient to keep temperature or humidity within recommended ranges. Furthermore, temperature profiles in commercial retail displays showed large variations depending on the location of the produce in the displays (Nunes, Emond, Rauth, Dea, & Chau, 2009). These surveys reinforce the fact that retail display cases represent a weak link in the maintenance of a proper cold chain management (Wells & Singh, 1989).

The highly variable temperature conditions associated with the storage of fresh-cut products in commercial open-refrigerated display cases dramatically affect the quality and safety of produce (Allende, Luo, McEvoy, Artés, & Wang, 2004; Jaxsens, Devlieghere, & Debevere, 2002; Nunes et al., 2009; Paull, 1999). Danyluk and Schaffner (2011) suggest that research-based time range estimates for retail storage and accurate correlations between time and temperature during retail storage are needed, and the importance of this lag time in modeling *E. coli* O157:H7 growth in leafy greens is currently unknown. Recently, Zeng, et al. (2014) reported that the profiles of long-term temperature abuse and high average temperature over time rather than transient temperature abuse, such as the defrost cycles of refrigeration units, lead to more accurate growth and an increased probability distributions of *E. coli* O157:H7 and *Listeria monocytogenes* growth during commercially-bagged salad greens transportation, retail storage and display. Despite these prior research efforts, important data gaps exist in the areas of temperature profile mapping of retail refrigerated display cases as impacted by case operating conditions, and their correlations with microbial growth and product quality; along with practical approaches that could generate real world management options. The aim of this study was to determine the spatial and temporal temperature profiles of the display cases under different operating conditions, and their association with the quality and microbiota of stored packaged baby spinach.

## 2. Materials and methods

### 2.1. Equipment and experimental operating conditions

Two 12-foot long retail display cases were installed in a room prepared exclusively for the cases with an HVAC system and ambient temperature control at 22 °C. The dimensions of the room are 3.8 m (L) × 3.6 m (W) × 2.4 m (H). Each case consisted of three 4-foot sections (from side to side) and five modular shelves (from top to bottom) that are with flexible placement. Each 4-foot section was installed with 6 columns of TRION Wonderbar™ tray shelves (Trion Industries, Inc. Wilkes-Barre, PA, USA). The TRION Wonderbar shelves had proprietary spring-loaded ‘push-shelving’ to accommodate the bagged products

and each column held up to 6 (30 cm × 23 cm) bags. External stand-alone condensing units provided customizable duty schedules for defrost time. Commercially packaged baby spinach leaves (170 g per bag; 30 cm × 23 cm bag) within 2 d of processing were donated by Dole Fresh Vegetables, Inc. (Bessemer City, NC, USA). The products were shipped overnight via commercial refrigerated truck (2–4 °C) to the Beltsville Agricultural Research Center at the US Department of Agriculture-Agricultural Research Services (Beltsville, MD, USA). All products tested in each trial were obtained from the same production line and shift in order to minimize the biological variation of the samples. Upon receiving, the samples were immediately transferred to a 1 °C cold-room where Model Trix-8 Log Tag temperature data loggers were installed (MicroDAQ, Contoocook, NH) inside and outside each bag (2 loggers per bag). The monitoring frequency was set at 2-min intervals and the samples were then loaded onto the display cases. All un-occupied spaces in the case were loaded with package simulators constructed using standard 1-gallon Ziploc® bags containing 65 g of shredded sponge material saturated with 266 mL of chlorine solution prepared at a ratio of bleach: water solution (7.5 g/L). In a preliminary study, we validated the response of these simulators to temperature changes compared to packaged baby spinach samples and found no significant differences ( $P > 0.05$ ).

### 2.2. Evaluation of temperature profiles, product quality and microbiota

Two duty schedules (thermostat, defrost time and duration) were tested. The first set of experiment had the thermostat set at 28 °F (−2.2 °C), with a 12 h defrost interval, each at 30 min duration, and the second experiment had the thermostat setting increased to 31 °F (−0.5 °C), while the defrost cycle remained the same (30 min, 12 h interval). In both set of experiments, commercially packaged baby spinach bags were loaded onto three adjacent columns and four shelves. Each column in each of the four shelves were filled with 6 spinach packages each, with a total of 72 (3 columns × 4 shelves × 6 bags) bags (Fig. 1). In both sets at time zero (product as received) and after three days of display storage, packages were opened and product was evaluated for tissue electrolyte leakage, overall quality, total aerobic mesophilic bacteria and psychrotrophic bacterial populations.

Sensory attributes of overall visual quality were evaluated immediately after opening the bags by a three-member trained panel following a modified procedure from (Luo, McEvoy, Wachtel, Kim, & Huang, 2004). Overall quality was evaluated with a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely (Meilgaard, Civille, & Carr, 1991).

Tissue electrolyte leakage was measured following a modified procedure from Luo et al. (2009). The samples (25 g) were submerged in 500 mL aliquots of distilled water at 5 °C for 30 min. The electrical conductivity of the solution was measured using a conductivity meter (model 135A; Orion Research Inc., Beverly City, Mass. USA). Total sample conductivity was determined for the same treatments after freezing at −20 °C for 24 h and subsequent thawing. Tissue electrolyte leakage was expressed as a percentage of the total conductivity.

Samples (25 g) of spinach leaves were randomly taken from each package, and macerated with 225 mL phosphate buffered saline in filtration stomacher using a Lab Stomacher (Biomaster 400, Seward, Ltd., London, UK) at 230 rpm for 2 min. A 50 µL sample of each filtrate or its appropriate dilution was spread on agar plates with an automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., West Yorkshire, U.K.). Enumeration of the total aerobic mesophilic bacteria and psychrotrophic bacteria were completed after plating



**Fig. 1.** Photographs of the open-refrigerated retail display case layout (Left) and salad and dummy-bag placement on the case (Right). Labels A to F indicate different columns, S1 to S4 represents different shelves, and D1 to D6 means different depths. Spinach bags were placed in columns B, C and D, shelf 1 through 4, and depth 1 through 6.

on tryptic soy agar (TSA, Difco Lab, Sparks, Md., USA.), and the incubation conditions for aerobic mesophilic bacteria were 28 °C for 2 days and 5 °C for 7–10 days for psychotropic bacteria. Microbial colonies were counted using an automated colony counter (ProtoCOL SR; Synoptics, Cambridge, UK) and reported as log CFU/g of tissue.

### 2.3. Options for minimizing temperature variation in commercial display case

Foam boards (20 cm × 15 cm × 1 cm) were used in different case-locations to reduce the heat exchange between the refrigerated air and the ambient air from the aisle. Four options were tested: (1) Front: The insulation foam boards were placed in front of the bags located on the first rows of the case; (2) Back: The insulation foam boards were placed in the back of the bags located in the last row of the case; (3) Front and Back: Insulation foam boards were placed both in the front and back of the display case; (4) Control: No insulation boards were placed. All salad bags contained internal and external data loggers.

### 2.4. Experimental design and statistical analysis

The experiments were conducted using factorial designs, with case shelf and depth as main factors. At least three preliminary studies were conducted before this reported final experiment. Preliminary studies showed minimum among-column temperature variations and therefore data from each column served as replicates for data analysis. Temperature data were recorded every 2 min and there were at least 720 temperature data points for each sample position. Data were analyzed according to a general linear model using PROC MIXED (SAS Institute Inc., Cary, NC). For all data, when effects were statistically significant, means were compared using Sidak adjusted *P*-values ( $P < 0.05$ ).

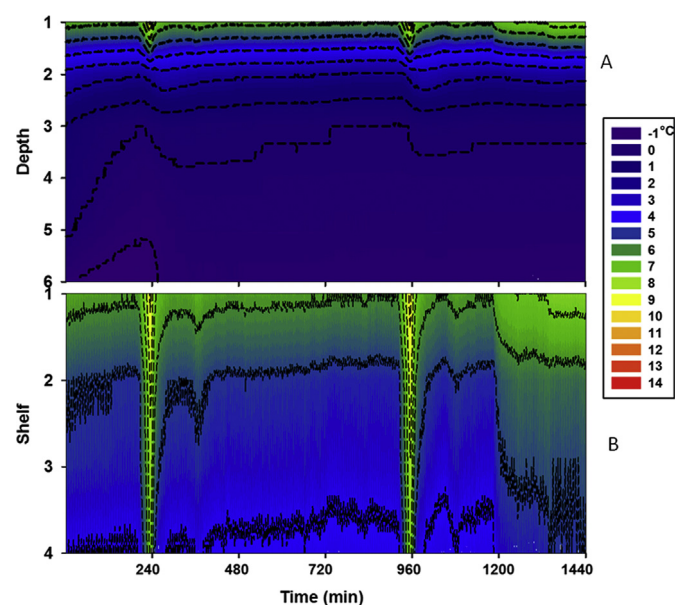
## 3. Results and discussion

### 3.1. Spatial and temporal temperature profiles under different operation conditions

The temperature of the bags on the display case shelf is a reflection of the display case operation settings and its spatial location within the case. With the thermostat setting at −2.2 °C and defrost cycle at 30 min with a 12 h interval, air temperatures

in all locations except for shelf 1 (Fig. 2A and B), were maintained below 5 °C during regular operations and during defrost cycles. However, substantial spatial temperature variations were found within the case. The largest and most significant ( $P < 0.01$ ) temperature differentials occurred among the depths (front vs. back), followed by shelves (top to bottom,  $P < 0.05$ ). As shown in Fig. 2A, samples located in depth 1 on shelf 1 (top shelf, positioned closest to the aisle) had the highest temperature (average 6.5 °C), whereas samples in depths 3–6 on shelf 1 (towards the rear of the case) had the lowest temperature (average −0.8 °C). The average temperature differential was 7.3 °C between depths 1 and 6 on shelf 1.

When comparing shelves 1–4, shelf 1 (top shelf) was the warmest, followed by shelves 2, 3 and 4 (Fig 2B). The warm temperatures in shelf 1 are likely due to warm ambient air convecting from the ceiling to maintain room temperature of 22 °C used to simulate actual retail conditions and the light fixtures located 0.45 m from the top of the case. Large pieces of insulation were placed



**Fig. 2.** Temperature profiles of packaged baby spinach bags located at shelf 1, depth 1–6 (A), and depth 1, shelves 1–4 (B). The case was operated under 28 °F (−2.2 °C) thermostat setting, and 30 min defrost cycles at 12 h interval.



on top of the case to prevent radiation heat from the lamps on top of the cases.

A significant ( $P < 0.001$ ) temporal effect was also noted on samples located at all positions. With the current defrosting occurring every 12 h, significant ( $P < 0.01$ ) temperature spikes were noted every 12 h, matching the defrost cycle schedule. There was a slight delay in temperature spike after the onset of defrost cycle, and this tails off after the defrost cycle ends. Also, the temperature spikes were more noticeable on samples located on front rows than those at the back.

### 3.2. Product quality and microbiota impacted by spatial and temporal temperature profiles

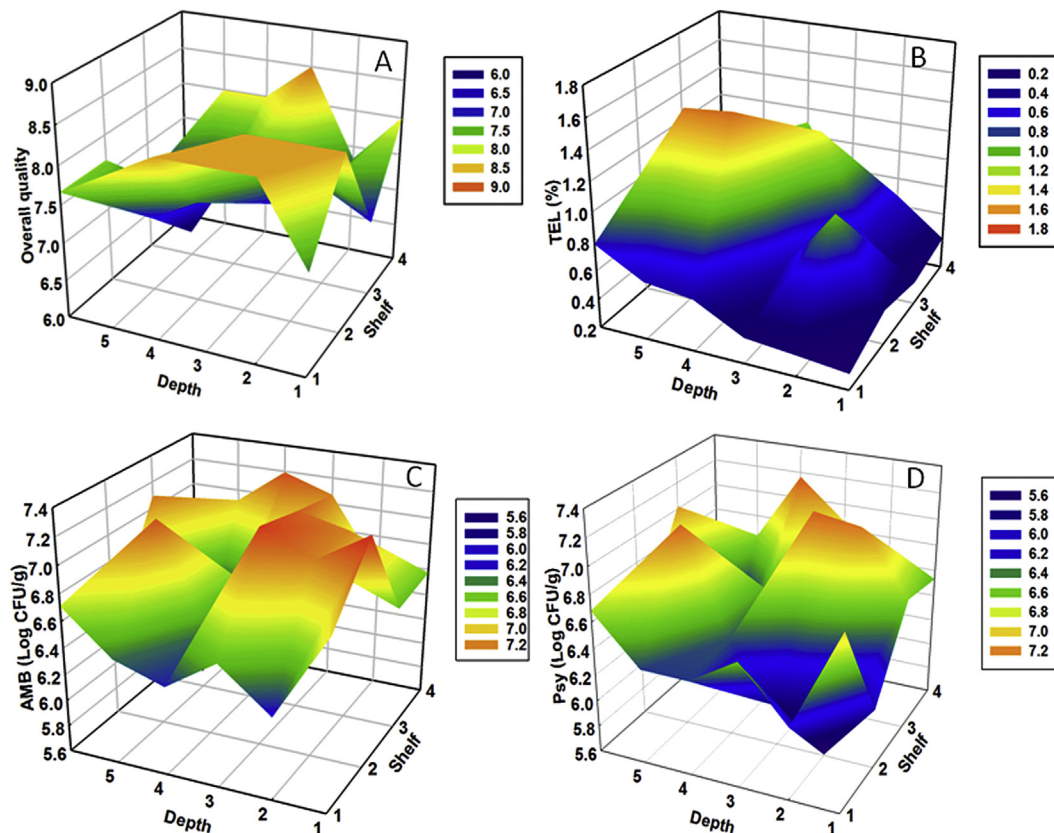
The visual appearance of the packaged baby spinach changed during storage based on their spatial positions (Fig. 3A). In shelf 1, at the  $-2.2\text{ }^{\circ}\text{C}$  thermostat setting, baby spinach samples showed a good visual appearance for depths 2 and 3, while samples in depths 1, 4, 5 and 6 had the lowest quality scores. Samples in depths 4, 5 and 6 showed signs of water soaking, which could be attributed to ice crystal formation and freeze damage (average  $-0.8\text{ }^{\circ}\text{C}$ ). Samples in depth 1 showed signs of yellowing, dehydration and decay due to the condensation caused by large temperature fluctuations, as well as the high temperature of the samples (average  $6.5\text{ }^{\circ}\text{C}$ ). The dehydration and yellowing (senescence) of depth 1 samples can be explained by the increased respiration rates, which led to even higher temperatures. Temperatures on depths 4–6 in shelf 3 were below  $0\text{ }^{\circ}\text{C}$ . These conditions impacted the quality of the baby spinach and the scores were lower than samples in other locations.

Kou et al. (2014) 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. They reported a similar phenomenon where baby spinach yellowing was caused by high storage temperature.

Temperature heterogeneity inside the display cases is consistent with previous results (Morelli, Noel, Rosset, & Poumeyrol, 2012). Morelli et al. (2012) reported 70% of time–temperature profiles were unsatisfactory where foodstuffs must remain at temperatures below or equal to  $7\text{ }^{\circ}\text{C}$ .

At the  $-2.2\text{ }^{\circ}\text{C}$  thermostat setting, the tissue electrolyte leakage (TEL) of spinach leaves was measured from day 0–3. As shown in Fig. 3B, for samples located in depth 1 to 6 on shelf 1, there was a gradual increase in tissue electrolyte leakage, which may be attributed to the decrease in temperature. Similar trends were also found on other shelves, except for samples in depth 2 on shelf 2, which had a slight increase; most likely due to sample variation. As the temperature decreased on all shelves (from depth 1 to depth 6), freeze damage of the baby spinach samples was more severe, damaging plant cell membrane integrity and resulting in higher electrolyte leakage. This observation coincided with the visual quality scores (Fig. 3A) in which samples stored toward the back of the shelf showed a rapid decline in quality. Therefore, changes in case design or alterations of the cooling cycles may need to be implemented to avoid this from happening in a commercial setting.

Aerobic mesophilic bacteria counts on the first day of placement for this thermostat setting, averaged  $6.2\text{ log CFU/g}$ . The average



**Fig. 3.** Tissue electrolyte leakage (A), visual quality (B), Populations of aerobic mesophilic bacteria (C) and psychrotrophic bacteria (D) of packaged spinach stored on a displayed case for 3 days. The case was operated under  $28\text{ }^{\circ}\text{F}$  ( $-2.2\text{ }^{\circ}\text{C}$ ) thermostat setting, and 30 min defrost cycles at 12 h interval. Visual quality was assessed by a panel of three trained judges using a 9-point hedonic scale, where 9 ~ like extremely, 7 ~ like moderately, 5 ~ neither like nor dislike, 3 ~ dislike moderately, and 1 ~ dislike extremely. Values are the means of four replications.

aerobic mesophilic bacteria counts increased by 1.1 log CFU/g during the three day storage due to the low temperature and short storage time (Fig. 3C). However, the counts of aerobic mesophilic bacteria varied largely among all bags, probably due to the combination of normal sample variation and the effect of storage temperature in each position. These findings are in agreement with the studies by Luo et al. (2009) and Babic, Roy, Watada, and Wergin (1996). Luo et al. (2009) surveyed commercially packaged spinach and reported that the total aerobic bacterial counts ranged from 4.0 to 8.3 log CFU/g and 3.4 to 8.1 log CFU/g from day 0 to two weeks later, respectively. Babic et al. (1996) evaluated the changes in microbial populations of fresh cut spinach from 6 to 10 log CFU/g.

Psychrotrophic bacterial populations on the first day of placement ( $-2.2^{\circ}\text{C}$  thermostat setting) averaged 6.1 log CFU/g. Like aerobic mesophilic bacteria, the psychrotrophic bacterial populations on baby spinach samples did not increase during three day storage in the display case, and with similar variations in populations among different positions within the display case (Fig. 3D). Similar trends in temperature response were observed by Babic et al. (1996) and Luo et al. (2009) on spinach samples stored at different temperatures in various package atmospheres.

Given the fact that many bags, especially those located towards the rear of the case were frozen, the thermostat setting was increased to  $-0.5^{\circ}\text{C}$  in a follow up experiment. As shown in Fig. 4A and B, the temperature of the bags was substantially higher than those held under the  $-2.2^{\circ}\text{C}$  setting. While this setting alleviated some of the freezing issues noted with the  $-2.2^{\circ}\text{C}$  thermostat setting, this setting also heightened the incidence of high temperature abuse. With this setting, significantly ( $P < 0.05$ ) more samples had temperature exceeding  $5^{\circ}\text{C}$  storage recommended by the FDA for TCS foods. However, similar to the  $-2.2^{\circ}\text{C}$  thermostat setting, there was a large spatial and temporal difference on samples stored at different locations ( $P < 0.01$ ).

For the  $-0.5^{\circ}\text{C}$  thermostat setting, we analyzed the tissue electrolyte leakage from the baby spinach samples, and determined the aerobic mesophilic bacteria population counts. For different shelves, samples on shelf 4, from depth 3 to 6, had levels of tissue electrolyte leakage that were higher than other shelves, as observed with the  $-2.2^{\circ}\text{C}$  thermostat setting. Samples located on

depths 1 and 6 had the highest relative tissue electrolyte leakage (Fig. 5A), as a reflection of the temperature extremes. Samples located on depth 1 were exposed to higher temperature abuse, resulting in an increased respiration rate and eventual senescence. Conversely, samples in depth 6 were exposed to colder air which resulted in a higher percentage of frozen samples that manifested in increased electrolyte leakage. All samples located on depth 1 of each shelf showed higher aerobic mesophilic bacteria counts, especially for shelves 1 and 4 (Fig. 5B). This most likely occurred because of the bag's proximity to the warm air coming from the aisle and the return.

### 3.3. Practical modifications to improve temperature uniformity in commercial display cases

A technical challenge of open display cases is to keep products in the front rows below  $5^{\circ}\text{C}$  and products in the back above freezing. Thermostat setting, room temperature and HVAC systems are factors that affect product temperatures inside the open case. Our observations are consistent with a study by Laguerre and co-workers who reported an increase in the air and load temperatures at the front of the display case as the room temperature was raised (Laguerre, Hoang, & Flick, 2012).

Although changing case duty cycle could increase or decrease product temperatures, lowering the case thermostat setting would lead to the potential for freezing damage, while increasing thermostat setting may increase the risk of high temperature abuse and violation of the FDA Food Code (Fig. 6A, Depth 1). Therefore, to address those issues, several options were tested using insulated foam blocks. As shown in Fig. 6B, placing foam insulation in front of the first rows of the product significantly ( $P < 0.05$ ) reduced the temperature by blocking the ambient hot air from entering the case. The sample temperature in the first depth position was about  $3.5^{\circ}\text{C}$  lower than those without the blocks (Fig. 6A, Depth 1). Similarly, placing the foam blocks in the back of the case (Fig. 6C) raised the temperature for those located in the back depth up to  $0.6^{\circ}\text{C}$  by blocking the cold air from directly reaching those bags. When insulated foam boards were placed to both the front and back of the case, these significantly ( $P < 0.05$ ) affected temperature uniformity by decreasing the temperature of bags in the first depth ( $3.5^{\circ}\text{C}$ ) and increasing the temperature of the product on the back row ( $0.5^{\circ}\text{C}$ ) (Fig. 6D). After placing foam blocks in the front and/or the back or both the insulation blocked the ambient hot air and the cold air from the back of the case. All these results indicate that a simple modification of the case could lead to significant ( $P < 0.05$ ) improvement in temperature uniformity throughout all shelves and depths, resulting in the compliance of FDA food code storage temperature without risking product freezing.

Large temperature variations within the display case have been previously reported (Evans et al., 2007; Willocx et al., 1994). To alleviate these problems related to temperature abuse and freezing damage, retailers have been frequently rotating the products from front to back and back to front. However, those practices are labor intensive and costly. Foam blocks are a good alternative to this problem, and we demonstrated their effective insulation in the display case, however, these may not be suitable for commercial display case installation. Presently, the number of retail stores installing night curtains or transparent doors is increasing. The installation of curtains and doors would not only decrease the amount of warm air coming in from the aisle, but also retain the cold air coming from the back of the display case, and thus reduce energy costs. Studies conducted by the U.S. Department of Energy (DOE) showed that for open refrigerated display cases, infiltration loads comprise 70–80% of the total case heat load. For comparison, in display cases manufactured with vertical transparent display

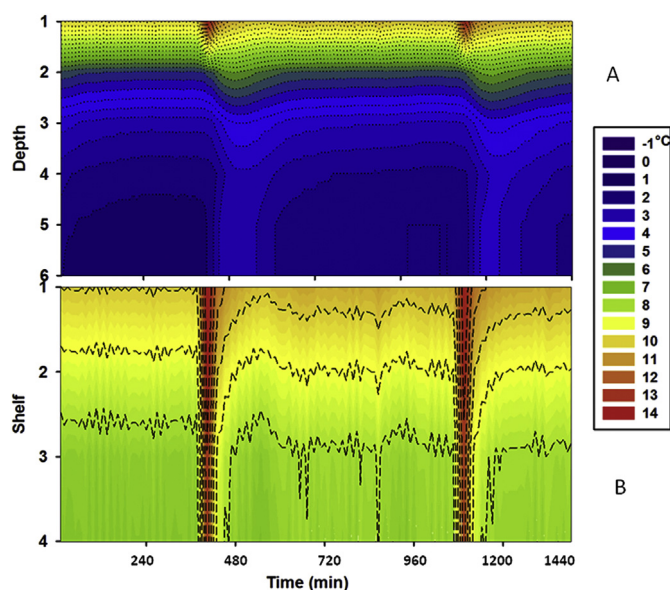
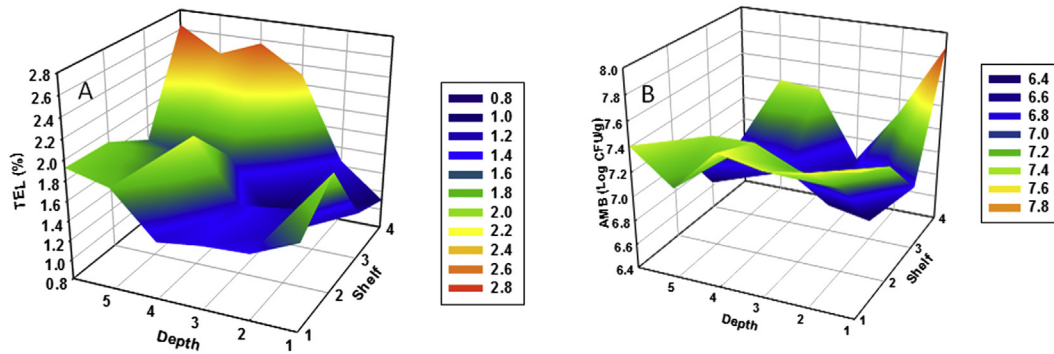


Fig. 4. Temperature profiles of packaged baby spinach bags located at shelf 1, depth 1–6 (A), and depth 1, shelves 1–4 (B). The case was operated under  $31^{\circ}\text{F}$  ( $-0.5^{\circ}\text{C}$ ) thermostat setting, and 30 min defrost cycles at 12 h interval.



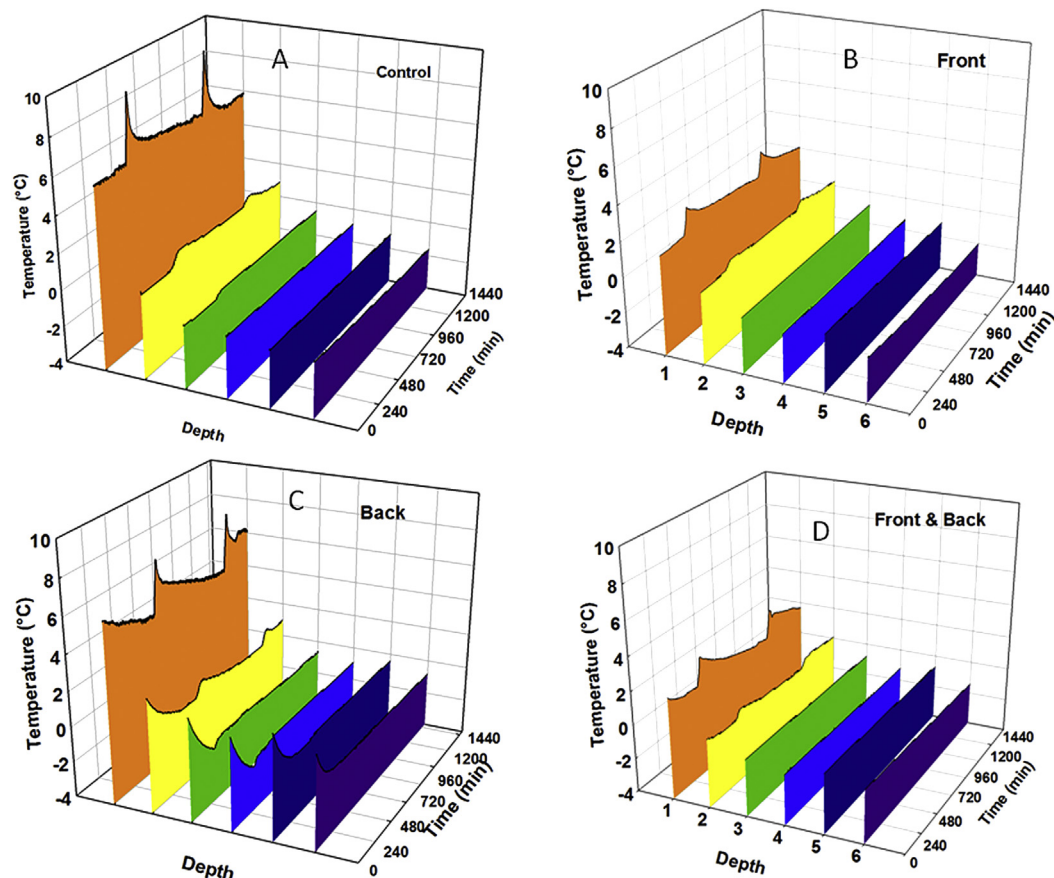
**Fig. 5.** Tissue electrolyte leakage (A) and population of aerobic mesophilic bacteria (B) of packaged spinach stored for 3 days. The case was operated under 31 °F (−0.5 °C) thermostat setting, and 30 min defrost cycles at 12 h interval. Values are the means of four replications.

doors that are operating in a similar configuration, the infiltration accounts for roughly 10% of the heat load to be removed from the display case (DOE, 2012). Results presented in this study indicate that by blocking the ambient air from entering the case, via curtains or doors, may also help improve temperature uniformity within the case, resulting in increased product quality.

#### 4. Conclusions

This research presented detailed thermograph mapping of spatial and temporal temperature profiles of a commercial open refrigerated display case. The changes in display case duty schedule on temperature profile and its associated effect on product quality

and microbiota were also evaluated. Thermostat setting of −2.2 °C thermostat, 12 h defrost interval for 30 min maintained the temperature below 5 °C for the majority of the samples, thus facilitating the compliance with FDA Food Code recommendations for TCS foods. However, a large amount of baby spinach suffered freezing damage was noted in those bags located in the back of the case. The display case duty setting of −0.5 °C, 12 h defrost for 30 min abated the freezing problem, though it resulted in temperature abuse for samples located at the front rows. The effect of ambient temperatures and the relatively large temperature variation between samples located on the front rows and those at the back rows appear to be the major technical challenges hindering the compliance of FDA Food Code without freezing the products.



**Fig. 6.** Changes in temperature profiles by placing insulated foam blocks in front (B), back (C), and both front and back (D) of the bags. Samples without insulated foam blocks (Control) are presented in A. The case was operated under 31 °F (−0.5 °C) thermostat setting, and 30 min defrost cycles at 12 h interval. Values are the means of four replications.



Placing insulated foam blocks in front and back rows can decrease the temperature differential (about 3.5 °C), however the commercial suitability of foam blocks needs to be explored further. We also suggest that curtains or transparent display doors may minimize temperature fluctuations in the retail case. This type of modification would not only decrease the amount of warm air coming in from the aisle, but also retains the cold air coming from the back of the display case, thus reducing energy costs while maintaining product quality and safety.

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