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Effects of Storage Temperature and Packaging on Physiological and Nutritional Quality Preservation of Minimally Processed Spinach

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ABSTRACT

Improper storage conditions of minimally processed spinach decrease its acceptability and dietary selection in terms of nutritive value to human health. In the present study, effects of temperatures (4 and 10°C) and packaging materials [Polypropylene (PP), Polyvinyl Chloride (PVC), Low-Density Polyethylene (LDPE)] were investigated for determining the successful storage conditions of minimally processed spinach. Based on the results, chlorophyll a (0.550 mg g^{-1}), chlorophyll b (0.500 mg g^{-1}), total chlorophyll (1.050 mg g^{-1}), and total carotenoids (0.310 mg g^{-1}) were maintained by PP at 4 °C. The highest antioxidant capacity (74.14%I), and total phenolic content (183.75 mg 100 g^{-1} gallic acid equivalent) were also determined in PP packages. Visual quality showed the same behavior in all packages except for the control, and storage at 4°C was greatly beneficial in improving visual quality of minimally processed spinach. In addition, LDPE delayed the increase in weight loss (0.41%) and respiration rate (27.32 mL CO_2 kg^{-1} h^{-1}). PVC preserved vivid green color of spinach at 4 °C. Some undesirable results were obtained at 10 °C storage because of rapid quality losses. As a result, storage at 4 °C in PP packages is an effective method to improve postharvest life of minimally processed spinach.

Keywords: Antioxidant capacity, Chlorophyll, *Spinacia oleracea* L., Total carotenoid, Total phenolic content.

INTRODUCTION

Spinach (*Spinacia oleracea* L.) has gained popularity in the market as minimally processed or fresh-cut leafy vegetable in recent years. The main problems of minimally processed spinach are yellowing, and wilting (Gil and Garrido, 2020). Spinach is a highly perishable vegetable, which has a commercial shelf life of about two weeks at 4°C (Kakade *et al*., 2015). Aktsoglou *et al*. (2019) also stated that spinach is recommended to store at 5 and 10°C for 15 and 10 days, respectively. In another study, the shelf life of spinach was extended up to 8 days only when stored under refrigerator conditions (5±1°C) (Garande *et al*., 2019).

Spinach is either sold intact (unprocessed) or minimally processed in local markets, supermarkets, and retail markets. In respect to storage temperature, 50% of the domestic and 20% of the commercial refrigerator temperatures are higher than or equal to 4 (for refrigerated cabinets) and 10°C (for cool stores). These two temperature levels represent the marketing conditions of spinach. This research aimed to determine the storage losses of minimally processed spinach in different packaging materials in terms of quality and quantity during storage and to observe the efficacy of common storage temperatures on maintaining some quality parameters of spinach.

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MATERIALS AND METHODS

Plant Material

Commercial spinach cultivar 'Amador F_1' were harvested at a commercially mature stage (at 40 days after growing) at the end of November in 2020 from a commercial farm located at Beypazarı District of Ankara (40° 09' 38.9" N, 31° 55' 01.9" E, 700 m in elevation) in Turkey. After harvesting, mature leaves of uniform size were chosen and placed in small containers, and immediately transported to the Postharvest Laboratory.

Sample Preparation, Packaging, and Storage

The roots and undesirable leaves (damaged, withered, and yellowish) of the spinach were discarded with a sterile sharpened knife manually. For minimal processing, the leaves were washed with cold tap water and immersed in sodium hypochlorite solution (0.2 g L^{-1} of free chlorine) for 3 minutes and centrifuged in a manual centrifuge at 800×*g* for 1 minute. Subsequently, they were rinsed with distilled water for 1 minute and centrifuged again. Finally, samples were portioned as 300 g each and packed with LDPE (Low-Density Polyethylene, 36×24 cm size, 150 μm-thickness), thermally sealed by Packtech (PCS-200, 300W, 220/240V, 50/60 Hz), PP (transparent blister Polypropylene clamshell box, 29×21×4 cm size, 700 μm thickness), PVC (Polyvinyl Chloride, 12-μm-thick) stretch film wrapping in polystyrene foam trays (36×27×2 cm size). O_2 Transmission Rate (OTR), CO_2 Transmission Rates (CTR), and Water Vapor Transmission Rate (WVTR) of the selected films were as follows; OTR: 113, 43.2, and 1,380 [cm^3 (m^2 h ΔC$)^{-1}$] in LDPE, PP and PVC at 20°C temperature with 75% RH condition. CTR: 630, 200, and 8300 [cm^3 (m^2 h ΔC$)^{-1}$] in LDPE, PP and PVC at 20°C temperature with 75% RH condition. WVTR: 5, 3, and 30 g m^{-2}.day at 38°C and 90 % RH condition. Spinach (MA packed and non-packed) were stored at 4 and 10°C with 97±2% RH for 15 days. The analyses were performed every 3 days (0, 3, 6, 9, 12, 15 days).

Quality Evaluations

Weight Loss

Weight Loss (WL) was determined by weighing the same samples at the end of each storage period with a digital scale (Mettler Toledo, USA) and calculated as a percentage (%).

Respiration Rate

Respiration Rate (RR) of spinach was determined according to the closed atmosphere method (Klein and Lurie, 1990). For this purpose, each replication (300 g) of treatment including the control was placed in sealed glass jars for 2 hours, and then the amount of CO_2 release from leaves was measured digitally with a CO_2 analyzer (Servomex PA404). Results were calculated using the weight and volume of the leaves and RR was given as mL CO_2 kg^{-1} h^{-1}.

Leaf Color

Leaf color was measured using a Minolta CR-400 colorimeter (Osaka, Japan). Changes of leaf color were quantified on the CIE L*, a*, b* color space system while Hue angle (h°) [(h°= 180+$\tan^{-1}$ (b*/a*)] and Chroma values [C*= $(a^{*2} + b^{*2})^{1/2}$] were calculated from *a** and *b** values.

Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Total Carotenoid

Chlorophyll a (Chl a) and Chlorophyll b (Chl b) were measured according to the method described by Witham *et al*. (1971). Total carotenoid (Tc) was determined based on the method of Kirk and Allen (1965). To measure Chl a and Chl b, 0.25 g of previously frozen spinach leaves were homogenized with 10 mL acetone (Merck) (80%) by a homogenizer (IKA-Labortechnik, Ultra-turrax T25) and filtered through Whatman No. 42 filter paper and, then, the volume was adjusted to 25 mL with acetone (80%). Chl and Tc contents were determined by an automated UV–Vis spectrophotometer (Shimadzu, Japan) according to the equations given below:

Chl a (mg g^{-1})= 12.7 (A663)–2.69 (A645) (1)

Chl b (mg g^{-1})= 22.9 (A645)–4.68 (A663) (2)

Total Chl (mg g^{-1})= Chl a+Chl b (3)

Tc (mg g^{-1})= A480+0.114 (A663)–0.638 (A645) (4)

Where A= Absorbance, Chl a= Chlorophyll a, Chl b= Chlorophyll b, Tc= Total carotenoid.

Visual Quality

Visual Quality (VQ) was scored according to the methods of Medina *et al.* (2012) and Mudau *et al*. (2018) with partial modifications. VQ was evaluated by six trained panel members with equal gender ratio, ages ranging between 25–40 years old, considering appearance, color, brightness, uniformity, and freshness following a 9-point Hedonic scale where, 9= ‘‘Excellent’’; 1= ‘‘Extremely poor’’; 5= ‘‘Fair’’. Scores below 5 were considered as restriction levels for marketability indicating the end of storage life.

Antioxidant Capacity and Total Phenolic Content

Antioxidant Capacity (AOC) and Total Phenolic Content (TPC) analyses were determined as in Karaca and Velioglu (2014) with some modifications. Briefly, 20 mL methanol (80:20, v/v) was added to 5 g fresh sample in 50 mL falcon tube, then, homogenized at 9,500×*g* for 1.5 minutes. The homogenate was shaken with a mechanical shaker for 30 min and the mixture was centrifuged at 14,000×*g* for 15 minutes. The supernatant was separated and filtered by a 0.45 μm membrane filter (Millipore, USA). The prepared extract was used for both AOC and TPC analyses.

AOC was measured by considering the principles of Brand-Williams *et al.* (1995) with 2,2-Diphenyl-1picrylhydrazyl (Sigma, D9132) (DPPH) and calculated as percentage of Inhibition (I%):

$$\text{Inhibition (I\%)}= ((Abs_{control}-Abs_{sample})/Abs_{control})\times 100 \quad (5)$$

TPC was detected using a spectrophotometer (Shimadzu UV/VIS) by adhering to the methods of Singleton and Rossi (1965) with a partial modification. Briefly, 50 μL of the extract was transferred to a tube and mixed by adding 1,550 μL of distilled water and 100 μL of Folin Ciocalteu's phenol reagent. Then, this mixture was held at 20°C for 3 minutes. After adding 300 μL of Na_2CO_3 (20%) solution, the final mixture was kept in the dark for 1 h. The absorbance values of the samples were measured at 765 nm with a spectrophotometer. TPC results were expressed as mg 100 g^{-1} GAE (Gallic Acid Equivalent) fresh weight.

Statistical Analysis

Storage Periods (SPs), Storage Temperatures (STs), and Packaging Materials (PMs) were considered as variables. The study was a factorial experiment based on a completely randomized design with three replications. Each package (300 g) was considered as one replication. Data were analyzed by two-way Variance Analysis (ANOVA) using MINITAB 17 (Trial version) program at $P\leq 0.05$ significance level. Tukey’s test in MSTAT-C was used to control the significant differences at $P\leq 0.05$ levels. The results were presented as means with standard error of means.

RESULTS AND DISCUSSION

Weight Loss (WL) was influenced by the interaction of Storage Periods (SPs)×Storage

Temperatures (STs)×Packaging Materials (PMs) (P≤ 0.05) as shown in Table 1. Longer SPs caused a higher WL at both temperatures for all groups, which was consistent with the previous report (Kakade *et al*., 2015). A remarkable increase was observed in the controls at 10 over 4°C. According to the results, WL was lower in LDPE (0.41%, on average), followed by PP (0.43%, on average), PVC (0.98%, on average), and control (34.27%, on average) at 4°C, which were 0.83, 1.03, 1.91, and 37.78% in LDPE, PP, PVC, and the control, respectively, at 10°C at the end of the storage period (Figure 1). Overall, the current experiment revealed that LDPE was the most effective to reduce WL. In a report conducted by Garande *et al*. (2019), WL was determined as 8.54 and 9.64% in PE bags at 5 °C on the 6^{th} and 8^{th} days of the storage. Meanwhile, the results of WL were lower than the findings of Mudau *et al*. (2018), who stored spinach in MAP at 4 and 10°C for 12 days and 7 days, respectively. Spinach quality is particularly sensitive to WL, with 3% water loss, making this commodity unmarketable (Bartz and Brecht, 2002). In accordance with this, the WL of spinach in MAP remained below 3% during the entire storage period at 4 and 10°C in the study. According to the findings, besides the factors mentioned above, one possible reason for WL in spinach is related to some independent factors such as packaging material, and storage period as consistent with Kaur *et al*. (2011).

The Respiration Rate (RR) of spinach stored at different temperatures for the

Table 1. Significant effects of Storage Periods (SPs), Storage Temperature (STs), and Package Materials (PMs) on some quality parameters of minimally processed spinach.[a]

Significant Effects	WL	RR	h°	C*	Chl a	Chl b	Total Chl	Tc	AOC	TPC
SPs	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
STs	0.069	0.025	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.072
PMs	0.000	0.012	0.001	0.074	0.000	0.000	0.000	0.013	0.020	0.031
SPs×STs	0.000	0.147	0.007	0.000	0.000	0.000	0.000	0.004	0.000	0.113
SPs×PMs	0.000	0.214	0.000	0.003	0.000	0.000	0.000	0.000	0.134	0.635
STs×PMs	0.000	0.526	0.034	0.440	0.000	0.012	0.049	0.229	0.228	0.526
SPs×STs×PMs	0.000	0.685	0.574	0.791	0.000	0.000	0.000	0.000	0.847	0.588

[a] WL: Weight Loss, RR: Respiration Rate, h˚: Hue angle, C*: Chroma, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Total Chl: Total Chlorophyll, Tc: Total Carotenoid, AOC: Antioxidant Capacity, TPC: Total Phenolic Content.

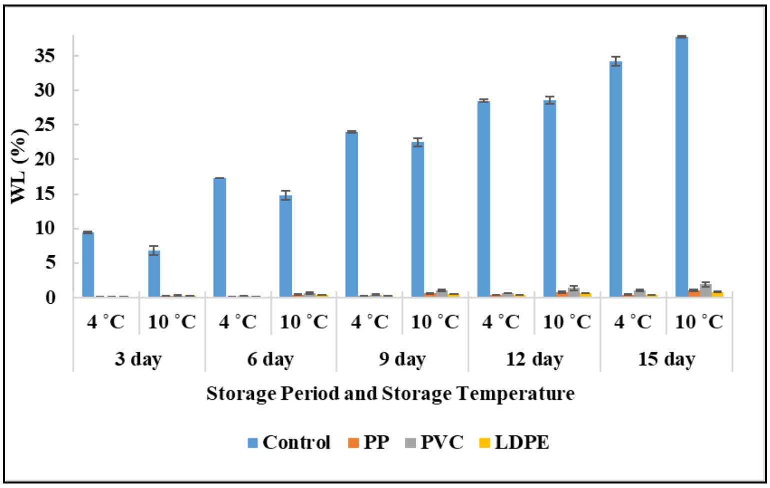

Figure 1. Effect of temperature and different packaging materials on Weight Loss (WL) of minimally processed spinach during storage.

different packaging materials is shown in Table 2. Initial RR of samples was recorded as 33.65 mL CO_2 kg^{-1} h^{-1} and this parameter exhibited fluctuations throughout the storage period. A significant increase in the RR of samples was recorded after three days of storage (40.03 mL CO_2 kg^{-1} h^{-1}), then, slightly increased on the 6th day (44.68 mL CO_2 kg^{-1} h^{-1}), and, then, decreased gradually to 15.94 mL CO_2 kg^{-1} h^{-1} at the end of the storage (Table 2). This behavior is highly consistent with the results of Mersinli *et al*. (2021) who stored minimally processed spinach for 25 days. The RR of samples stored at 4°C (27.58 mL CO_2 kg^{-1} h^{-1}) was lower than 10°C (32.66 mL CO_2 kg^{-1} h^{-1}). The higher RR at high temperatures could be explained by the fact that temperature has a direct impact on partial pressure (Pereira *et al*., 2017). Similar observations were reported by Singh *et al*. (2014) and Singla *et al*. (2020) in fresh baby corn and fresh black carrot, respectively. With regard to packaging materials, spinach packed in PVC had higher RR as 30.65 mL CO_2 kg^{-1} h^{-1} (Table 2) and the control (34.10 mL CO_2 kg^{-1} h^{-1}). This might be due to the high permeability of PVC film to respiratory gases (Lima *et al*., 2019). On the other hand, LDPE gave the best results in terms of retarding the increase in RR of minimally processed spinach.

According to Table 3, initially, color of the samples was dark green depending on h° value of 127.07°. The h° value decreased with prolonging storage period in all groups but this decrease was more noticeable at 10°C (125.76°, on average) than 4°C (127.18°, on average), presumably due to an increase in senescence. These results showed that storage at 4°C inhibited yellowing in minimally processed spinach, and storage at 10°C had a negative effect on spinach color, demonstrating that it caused chlorophyll degradation. Much evidence suggesting that the lowest yellowing rate may be due to high humidity and low temperature confirmed similar findings with the current results on spinach (Garande *et al*., 2019). Additionally, similar results were obtained from a previous study carried out by Hodges *et al*. (2000). By the way, the obtained green color results are higher than Viškelis *et al*. (2015) who found h° as 111° and 112° in PE and PP, respectively, at 4°C for 9 days. This may be due to a difference

Table 2. Respiration Rate (RR) and Total Phenolic Content (TPC) values of minimally processed spinach during storage at 4 and 10°C using different packaging materials.[a]

Factors	RR (mL CO_2 kg^{-1} h^{-1})	TPC (mg $100g^{-1}$ GAE)
SPs		
0th Day	33.65 ± 2.12 C*[a]	176.47 ± 2.74 C
3rd Day	40 03 ± 3.21 B	193.50 ± 1.93 AB
6th Day	44.68 ± 2.14 A	197.60 ± 1.79 A
9th Day	28.71 ± 1.18 D	180.78 ± 5.42 BC
12th Day	17.73 ± 2.47 E	156.79 ± 3.51 D
15th Day	15.94 ± 1.47 E	154.42 ± 3.76 D
STs		
4˚C	27.58 ± 2.45 B*[b]	179.07 ± 2.64 ns*[c]
10˚C	32.66 ± 1.58 A	174.12 ± 2.84 ns
PMs		
Control (-MAP)	34.10 ± 2.15 A*[d]	174.60 ± 4.90 B
PP	28.41 ± 3.89 C	183.75 ± 3.17 A
PVC	30.65 ± 1.74 B	173.93 ± 3.88 B
LDPE	27.32 ± 2.74 D	174.10 ± 3.28 B

[a] Capital letters show differences among storage periods, [b] Capital letters show differences among storage temperatures, [c] ns: Non-significant, [d] Capital letters show differences among packages at P≤ 0.05 error level according to Tukey's test. * Mean±Standard error of mean.

Table 3. Hue angle **(**h˚), Chroma (C*), and Antioxidant Capacity (AOC) values of minimally processed spinach during storage at 4and 10°C using different packaging materials.

Factors	h˚	C*	AOC (%I)
SPs			
0th Day	127.07 ± 0.42 ABC* [a]	17.95 ± 0.49 BC	79.63 ± 0.12 A
3rd Day	128.68 ± 0.49 A	16.92 ± 0.38 C	85.16 ± 1.02 A
6th Day	127.75 ± 0.30 AB	16.20 ± 0.26 C	82.41 ± 1.45 A
9th Day	126.52 ± 0.67 BC	16.97 ± 0.53 BC	71.78 ± 2.46 B
12th Day	125.46 ± 0.72 C	18.77 ± 0.59 B	59.06 ± 2.95 C
15th Day	123.33 ± 0.74 D	22.09 ± 0.81 A	46.73 ± 2.95 D
STs			
4˚C	127.18 ± 0.29 A* [b]	17.23 ± 0.27 B	74.53 ± 1.32 A
10˚C	125.76 ± 0.44 B	19.07 ± 0.44 A	67.06 ± 2.46 B
PMs			
Control (-MAP)	125.38 ± 0.82 C*[c]	18.75 ± 0.77 ns*[d]	68.14 ± 3.41 B
PP	127.02 ± 0.34 AB	17.72 ± 0.43 ns	74.14 ± 2.70 A
PVC	127.49 ± 0.48 A	17.64 ± 0.42 ns	69.72 ± 2.68 AB
LDPE	125.99 ± 0.35 BC	18.48 ± 0.46 ns	71.17 ± 2.60 AB
SPs×STs			
0th Day×4˚C	127.07 ± 0.61 AB,a*[e]	17.95 ± 0.71 AB,a	79.63 ± 0.17 AB,a
3rd Day×4˚C	129.14 ± 0.85 A,a	17.16 ± 0.65 ABC,a	84.56 ± 1.08 A,a
6th Day×4˚C	127.29 ± 0.40 AB,a	15.96 ± 0.32 BC,a	83.44 ± 1.77 AB,a
9th Day×4˚C	127.90 ± 0.50 A,a	15.36 ± 0.23 C,b	74.50 ± 2.66 BC,a
12th Day×4˚C	126.55 ± 0.91 AB,a	17.39 ± 0.48 ABC,b	65.97 ± 2.25 CD,a
15th Day×4˚C	125.12 ± 0.53 B,a	19.55 ± 0.71 A,b	59.06 ± 2.06 D,a
0th Day×10˚C	127.07 ± 0.61 AB,a	17.95 ± 0.71 BC,a	79.63 ± 0.17 A,a
3rd Day×10˚C	128.22 ± 0.51 A,a	16.67 ± 0.41 C,a	85.76 ± 1.77 A,a
6th Day×10˚C	128.21 ± 0.43 A,a	16.43 ± 0.41 C,a	81.39 ± 2.35 A,a
9th Day×10˚C	125.15 ± 1.13 B,b	18.57 ± 0.83 BC,a	69.06 ± 4.11 B,a
12th Day×10˚C	124.37 ± 1.06 B,a	20.15 ± 0.95 B,a	52.14 ± 4.77 C,b
15th Day×10˚C	121.53 ± 1.21 C,b	24.64 ± 1.06 A,a	34.39 ± 2.10 D,b
SPs×PMs			
0th Day×Control	127.07 ± 0.91 ABa*[f]	17.95 ± 1.05 Ba	79.63 ± 0.26 ns
0th Day×PP	127.07 ± 0.91 Aa	17.95 ± 1.05 ABa	79.63 ± 0.26 ns
0th Day×PVC	127.07 ± 0.91 ABa	17.95 ± 1.05 Aa	79.63 ± 0.26 ns
0th Day×LDPE	127.07 ± 0.91 ABa	17.95 ± 1.05 Ba	79.63 ± 0.26 ns
3rd Day×Control	130.53 ± 1.49 Aa	15.64 ± 0.59 Ba	84.08 ± 1.81 ns
3rd Day×PP	128.63 ± 0.80 Aa	17.46 ± 1.10 Ba	84.65 ± 2.89 ns
3rd Day×PVC	128.05 ± 0.63 ABa	17.34 ± 0.40 Aa	84.10 ± 2.22 ns
3rd Day×LDPE	127.51 ± 0.49 Aa	17.23 ± 0.69 Ba	87.81 ± 0.79 ns
6th Day×Control	128.36 ± 0.67 ABa	14.92 ± 0.36 Ba	86.82 ± 0.74 ns
6th Day×PP	128.37 ± 0.39 Aa	16.04 ± 0.34 Ba	84.23 ± 1.54 ns
6th Day×PVC	127.86 ± 0.70 ABa	16.89 ± 0.45 Aa	76.30 ± 4.68 ns
6th Day×LDPE	126.41 ± 0.30 ABa	16.94 ± 0.52 Ba	82.30 ± 1.46 ns
9th Day×Control	124.67 ± 2.22 BCb	17.07 ± 1.57 Ba	65.71 ± 6.58 ns
9th Day×PP	126.81 ± 0.56 Aab	16.34 ± 0.90 B,a	80.42 ± 2.30 ns
9th Day×PVC	128.44 ± 1.19 ABa	16.68 ± 0.85 Aa	70.83 ± 4.79 ns
9th Day×LDPE	126.19 ± 0.42 ABab	17.79 ± 1.05 Ba	70.18 ± 4.12 ns
12th Day×Control	121.07 ± 1.13 CDb	21.79 ± 1.47 Aa	49.58 ± 7.00 ns
12th Day×PP	126.45 ± 0.61 Aa	17.44 ± 0.67 Bb	67.67 ± 7.80 ns
12th Day×PVC	128.76 ± 1.13 Aa	17.09 ± 0.91 Ab	59.55 ± 3.39 ns
12th Day×LDPE	125.54 ± 0.63 ABa	18.75 ± 0.63 ABab	59.43 ± 2.51 ns
15th Day×Control	120.57 ± 1.71 Db	25.17 ± 1.80 Aa	43.03 ± 6.92 ns
15th Day×PP	124.76 ± 0.72 Aa	21.11 ± 1.05 Ab	48.28 ± 5.47 ns
15th Day×PVC	124.76 ± 1.84 Ba	19.89 ± 1.73 Ab	47.94 ± 7.39 ns
15th Day×LDPE	123.21 ± 1.05 Bab	22.20 ± 1.39 Aab	47.65 ± 4.90 ns

[a] Capital letters show differences among storage periods, [b] Capital letters show differences among storage temperatures, [c] Capital letters show differences among packages, [d] ns: non-significant; [e] Capital letters show differences among storage periods in each storage temperature, lower letters show differences between storage temperatures in each storage period, [f] Capital letters show differences among storage periods in each package, lower letters show differences between packages in each storage period, [g] Capital letters show differences among packages in each storage temperature, lower letters show differences among storage temperature in each package at P≤ 0.05 error level according to Tukey's test. * Mean±Standard error of mean.

Table 3 countinued…

Countinied of Table 3. Hue angle (h˚), Chroma (C*), and Antioxidant Capacity (AOC) values of minimally processed spinach during storage at 4and 10°C using different packaging materials.

Factors	h˚	C*	AOC (%I)
STs×PMs			
4˚C×Control	126.81 ± 0.84 Aa[*g]	17.41 ± 0.65 ns	73.10 ± 3.25 ns
4˚C×PP	126.87 ± 0.48 Aa	17.13 ± 1.35 ns	78.94 ± 2.42 ns
4˚C×PVC	128.44 ± 0.56 Aa	16.93 ± 0.56 ns	73.56 ± 2.03 ns
4˚C×LDPE	126.59 ± 0.31 Aa	17.44 ± 0.65 ns	72.51 ± 2.68 ns
10˚C×Control	123.95 ± 1.36 Bb	20.10 ± 0.41 ns	63.19 ± 5.86 ns
10˚C×PP	127.16 ± 0.48 Aa	18.31 ± 0.70 ns	69.35 ± 4.62 ns
10˚C×PVC	126.54 ± 0.74 Ab	18.35 ± 0.53 ns	65.89 ± 4.87 ns
10˚C×LDPE	125.39 ± 0.60 ABa	19.51 ± 0.69 ns	69.82 ± 4.51 ns

[a] Capital letters show differences among storage periods, [b] Capital letters show differences among storage temperatures, [c] Capital letters show differences among packages, [d] ns: non-significant; [e] Capital letters show differences among storage periods in each storage temperature, lower letters show differences between storage temperatures in each storage period, [f] Capital letters show differences among storage periods in each package, lower letters show differences between packages in each storage period, [g] Capital letters show differences among packages in each storage temperature, lower letters show differences among storage temperature in each package at P≤ 0.05 error level according to Tukey's test. [*] Mean±Standard error of mean.

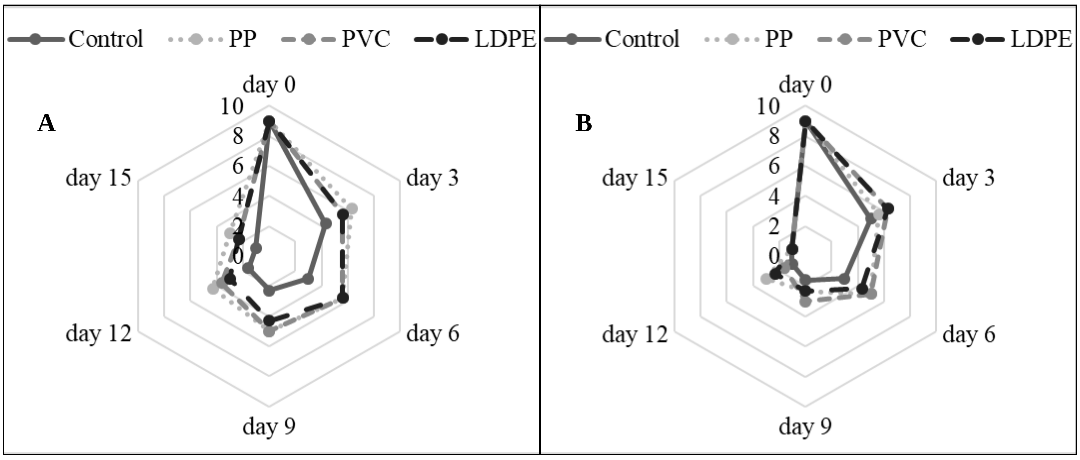

Figure 2. Effect of 4 (A) and 10°C (B) and different packaging materials on visual quality of minimally processed spinach during storage.

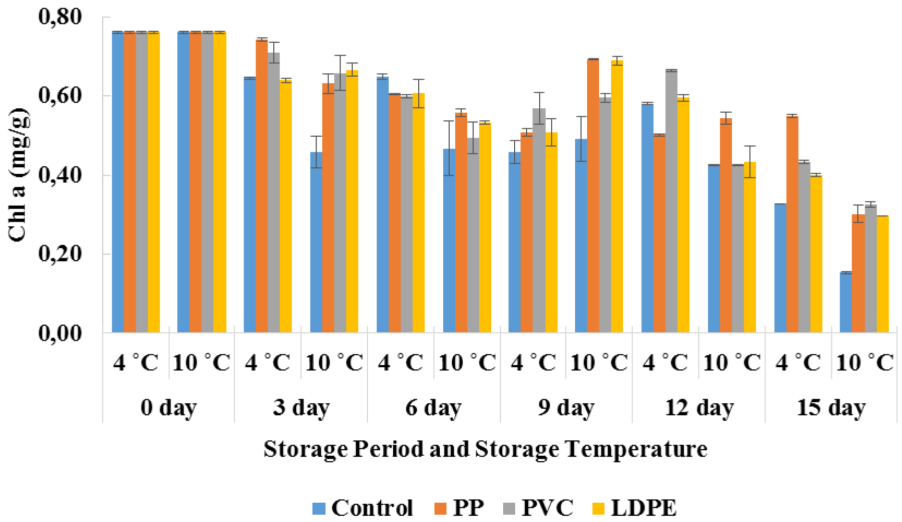

Figure 3. Effect of temperature and different packaging materials on Chlorophyll a (Chl a) of minimally processed spinach during storage.

in the package thickness. The highest h° values were obtained from PVC packed samples (128.44°, on average) stored at 4°C, while the lowest ones (123.95°) were determined in the controls at 10°C (Table 3). These findings for spinach storage agree with the study of Koukounaras *et al*. (2020) who stored spinach at 4°C for 12 days.

As shown in Table 3, during the entire storage period, the average C* values tended to increase and the same trend was observed in both temperatures. An increase of C* values is reflected in color parameters owing to chlorophyll degradation (Glowacz *et al*., 2013). Apart from this, samples stored at 10°C showed the highest values compared to those at 4°C. However, since there was no variation in the storage period among samples at 4°C, changes were significant at 10°C at the end of the storage. When the PMs were evaluated, no differences emerged in PVC and PP compared to the control and LDPE at the end of the storage period independently of the STs. These results exhibited that storage in PVC and PP materials maintained color vividness of minimally processed spinach leaves.

According to the results, Chl a and total chlorophyll were significant for the storage period in each package at 4 and 10°C, whereas significant changes were only found in Chl b at 10 °C (Table 1). Chl a content values ranged from 0.761 to 0.400 mg g^{-1} at 4°C and from 0.761 to 0.153 mg g^{-1} at 10°C (Figure 3). Chl a decreased with increasing storage period at both temperatures and this decrease was about 50% and 70% at 4 and 10°C, respectively. It is seen that 10°C did not have a protective effect on Chl a content of spinach, while 4°C contributed to preserving by delaying the degradation of Chl a. Among packaging materials, in particular, PP maintained Chl a content up to the last sampling day at 4°C.

Chl b content of spinach was 0.429 mg g^{-1} at the beginning, while it was almost stable at 4°C, a drastic reduction was seen in the control samples stored at 10°C up to 52% (Table 1). Chl b is catabolically transformed into Chl a before its degradation (Roca *et al*., 2004). Among storage periods in each package materials, the lowest value was determined in the control and LDPE at 10°C as 0.205 and 0.262 mg g^{-1} on average, respectively (Figure 4). A significant difference between temperatures in each group was only found in PP and controls at 4°C. Furthermore, storage at 4°C with PP was significantly effective in preserving Chl b content. Therefore, storage at 4°C in PP

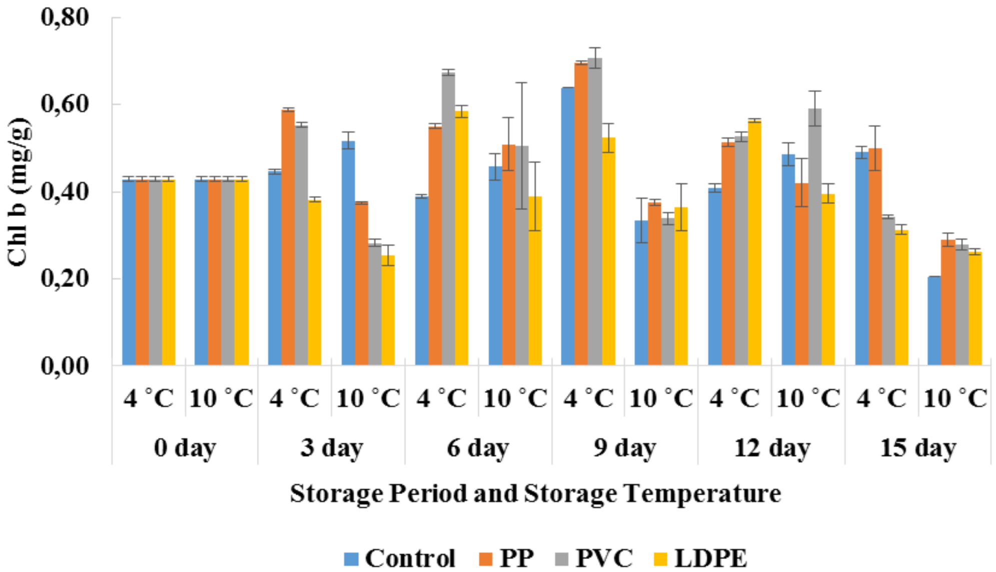

Figure 4. Effect of temperature and different packaging materials on Chlorophyll b (Chl b) of minimally processed spinach during storage.

could be the best way to keep higher Chl b content of spinach.

All of the dependent variables interactively affected total Chl (Chl a+Chl b) content (P= 0.000) throughout the storage as shown in Table 1. The lowest values were determined in LDPE (0.714 mg g^{-1}), PVC (0.775 mg g^{-1}), control (0.817 mg g^{-1}), and PP (1.050 mg g^{-1}) treatments at 4°C, which were 0.358, 0.559, 0.592, and 0.605 mg g^{-1} in the control, LDPE, PP, and PVC, respectively, at 10 °C at the end of the storage period (Figure 5). In accordance with the results reported by Spinardi *et al*. (2010) for the spinach, the content of total Chl was determined as 1.09 and 1.39 mg g^{-1} at 4 and 10°C after 6 days of storage. A significant reduction of total Chl content was recorded at 10 °C and showed a clear decrease on the 15th day in all groups. These results are in accordance with the earlier report on spinach (Aktsoglou *et al*., 2019). The decrease in chlorophylls could be explained by the development of yellowing. In terms of packaging materials, the storage of spinach in PP at 4°C performed significantly better than the other MAP's. The mechanism that drives beneficial chlorophyll retention phenomena is closely dependent on the lower O_2 transmission in-pack (Kaur *et al*., 2011).

Results in Figure 6 show that Total carotenoid (Tc) content generally tended to decrease regularly during the storage in all samples, which agrees with Mudau *et al*. (2015), indicating the carotenoid content in spinach decreases with increased storage time and degrades more rapidly after longer periods. However, these findings are inconsistent with Bergquist *et al*. (2006) who determined an increase of carotenoid in spinach during storage. In this study, Tc gradually declined from 0.416 to 0.189 mg g^{-1} at 4°C and from 0.416 to 0.207 mg g^{-1} at 10°C (Figure 6). There was no significant difference between storage temperatures. Further, PP-packed samples had the highest Tc values at 4°C and all MAPs helped to keep Tc at 10°C (Figure 6). Contrary to these findings, Spinardi *et al*. (2010) determined the Tc content as 57.29 and 57.01 mg g^{-1} at 4 and 10°C, respectively. This disparity may be related to genotypes, climatic conditions, or extraction methods.

As depicted in Figure 2, all samples started from superior Visual Quality (VQ) scores (9.0), which notably decreased at the end of the storage to around 1.5. The present findings are in concurs with the earlier report of Garande *et al*. (2019) on stored spinach. The higher VQ values were determined as average 4.73 for MAPs

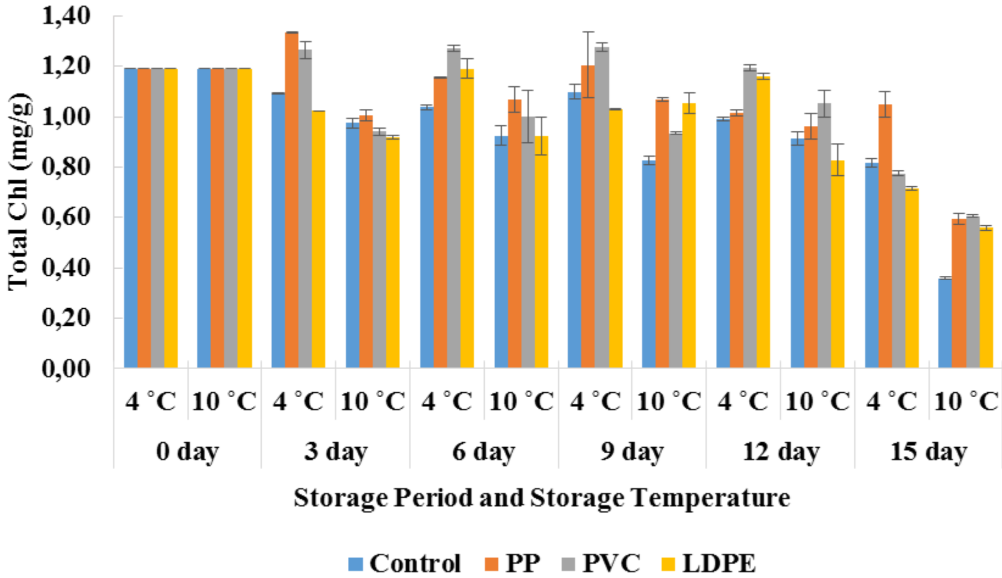

Figure 5. Effect of temperature and different packaging materials on Total Chlorophyll (Total Chl) of minimally processed spinach during storage.

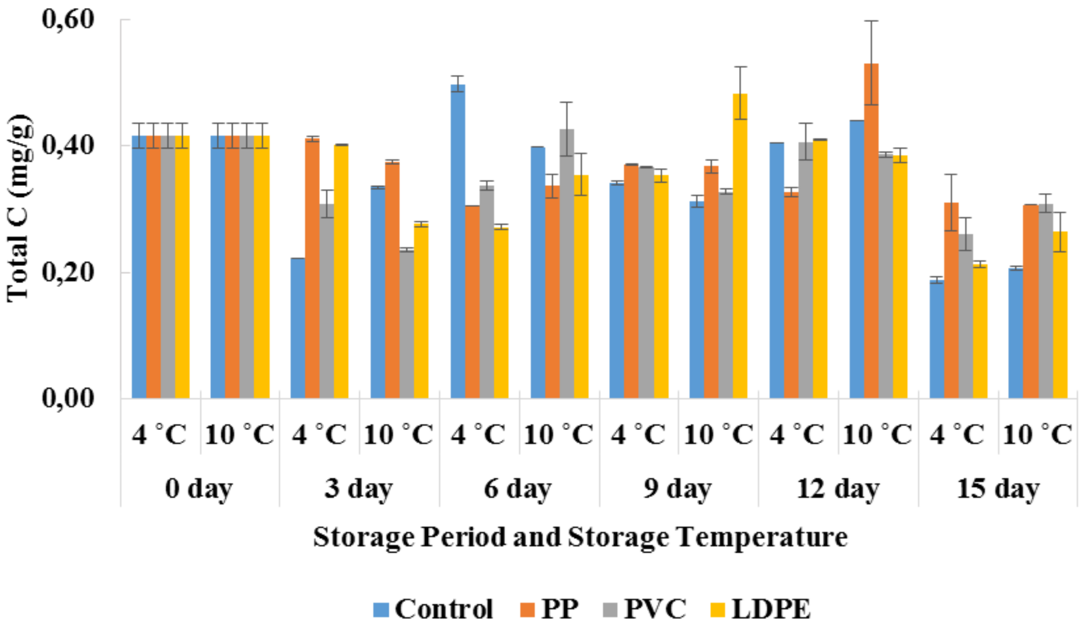

Figure 6. Effect of temperature and different packaging materials on Total carotenoid (Tc) of minimally processed spinach during storage.

compare to the controls (3.5). As expected, samples stored at 10°C (1.0, on average) showed signs of a rapid deterioration in VQ from all other treatments at 4°C (2.1, on average). In addition, clear differences were not observed in MAP samples at both temperatures along the storage period. According to the experience, this could be greatly dependent on multiple factors such as variety, growth stage, storage period, and temperature. Mudau *et al*. (2018) pointed out that a score of 5.0 for VQ could be a marker for the end of shelf life or marketability period in spinach. Based on these visual scores after 15 days of storage, spinach was considered commercially unacceptable after 6 days at 4 °C and 3 days at 10 °C, respectively, in MAPs in terms of showing yellowing, and decay.

Antioxidant Capacity (AOC) was determined as 79.63%I at initial, thereafter, rapidly decreased from 71.78%I (9th day) to 46.73%I (15thday) (Table 3). A decrease in AOC during storage is in accordance with a previous report of spinach under different storage conditions (Hodges and Forney, 2003). This decrease in samples stored at 10°C was higher (57%) than 4°C (26%). Similar findings were confirmed in the report by Mudau *et al*. (2017). Concerning PMs, the highest values (74.14%I) were observed in PP-packed samples all along with the storage. In some studies with similar conditions to this experiment, spinach stored in PE bags at 10°C, AOC content declined to half of the initial value after 3 days and declined faster after 7 days (Gil *et al*., 1999; Bergquist, 2006). The above decrease could be explained due to several compounds including amino acids, phenolic acids, flavonoids, and ascorbic acid as suggested by Mudau *et al*. (2017).

Total Phenolic Content (TPC) in samples increased on the 3rd day and sharply decreased after 9 days. Afterward, stabilization was seen in samples on the 12th and the 15th day (Table 2). Spinardi *et al*. (2010) have reported similar behavior in minimally processed spinach. Interestingly, we did not observe a significant effect of temperature on TPC. On the other hand, Aktsoglou *et al*. (2019) recorded lower TPC values (0.9-1.0 mg kg^{-1}) in stored spinach at 5 and 10°C than in this study. According to the experience, this difference may have been caused by genetic and ecological factors, and storage conditions. In addition, higher TPC values were found in PP compared to the control, LDPE, and PVC (Table 2). This may be because of the low O_2 transmission rate that prevails under stressful conditions. Kaur *et al*. (2011) determined the higher level of TPC in LDPE compared to PP, which is inconsistent with these experimental results. This could be attributed to MAP that has pronounced effects of metabolism on phenolics, depending on the variety or cultivar as indicated by Mudau *et al*. (2018).

CONCLUSIONS

According to the results, storage of minimally processed spinach at 4 °C maintained quality better than 10 °C. The results also revealed that MAP could maintain spinach quality during storage by keeping nutritional compounds and their benefits on health. All MAPs in this research significantly delayed changes in respiration rate and visual quality at 4°C. The combined effect of low storage temperature and PP package was more effective in maintaining the quality of chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, antioxidant capacity, and total phenolic content in stored samples. In addition, LDPE slowed down the increase in weight loss and respiration rate. PVC only enhanced color changes (Hue angle and Chroma) of spinach at 4°C. These results proved that different storage condition is the main driver of detrimental changes. Overall, based on visual quality scores and in terms of commercial acceptance, minimally processed spinach in MAP could be stored at 4°C for up to 6 days or at 10°C for up to 3 days. The findings of the present study will guide further studies designed to determine the most suitable MAP in minimally processed spinach.

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اثرهای دمای نگهداری و بسته بندی بر حفظ کیفیت فیزیولوژیکی و تغذیه‌ای اسفناج با فرآوری کمینه

س. آکان

چکیده

شرایط نگهداری نامناسب اسفناج با فرآوری کمینه باعث می شود که مقبولیت و انتخاب آن در رژیم غذایی از نظر ارزش غذایی برای سلامت انسان کاهش یابد. در این پژوهش، اثرهای دما (۴ درجه سانتیگراد و ۱۰ درجه سانتیگراد) و مواد بسته بندی شامل پلی پروپیلن (PP)، پلی وینیل کلراید (PVC)، و پلی اتیلن با چگالی کم (LDPE) برای تعیین شرایط نگهداری اسفناج با فرآوری کمینه بررسی شد. بر اساس نتایج، در تیمار PP در۴ درجه سانتی گراد، کلروفیل a (۰/۵۵۰ میلی‌گرم در گرم)، کلروفیل b (۰/۵۰۰ میلی‌گرم در گرم)، کلروفیل کل (۱/۰۵۰ میلی‌گرم در گرم) و کاروتنوئیدهای کل (۰/۳۱۰ میلی‌گرم در گرم) حفظ شدند. همچنین، بالاترین ظرفیت آنتی اکسیدانی (۷۴.۱۴٪ I) و محتوای فنل کل (۱۸۳.۷۵ میلی گرم در ۱۰۰ گرم در ۱ معادل اسید گالیک) در بسته های PP ثبت شد. کیفیت چشمی اسفناج وضعیت یکسانی را در همه بسته‌ها به جز تیمارشاهد نشان داد و نگهداری در دمای ۴ درجه سانتیگراد در بهبود کیفیت چشمی اسفناج با فرآوری کمینه بسیار مفید بود. علاوه بر این، LDPE افزایش اتلاف وزن (۰.۴۱٪) و نرخ تنفس (۲۷.۳۲ میلی لیتر CO_2 درکیلوگرم در ساعت) را به تاخیر انداخت. پی‌وی‌سی رنگ سبز اسفناج را در دمای ۴ درجه سانتیگراد حفظ کرد. برخی نتایج نگهداری در ۱۰ درجه سانتیگراد به دلیل تلفات سریع کیفیت، ناپسند بود. نتیجه این است که نگهداری در دمای ۴ درجه سانتیگراد در بسته بندی PP روشی موثری است برای بهبود انبارداری پس از برداشت اسفناج با فرآوری کمینه.