Response of baby spinach (Spinacia oleracea L.) to photoselective nettings on growth and postharvest quality.

HortScience 52(5):719–724. 2017. doi: 10.21273/HORTSCI11875-17

Response of Baby Spinach (*Spinacia oleracea* L.) to Photoselective Nettings on Growth and Postharvest Quality

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Additional index words. antioxidant activity, sensory quality, shade nets, flavonoids

***Abstract*. Manipulation of microenvironments by means of photoselective nettings is widely used to improve the productivity and quality of high-value vegetables. The aim of this study was to investigate the effect of photoselective nettings on growth, productivity, and postharvest quality attributes of baby spinach. Baby spinach cv. Ohio was grown from seeds, and the trial was repeated. Plants were planted in an open field (control) and under closed nets, viz., black, pearl, yellow, and red nets. At harvest, baby spinach leaves were subjected to 4, 10, and 20 °C storage temperatures for 12 days. Crops grown under black nets and stored at 4 °C retained higher level of antioxidant activity (0.23 g·kg^{-1}), whereas the least level of antioxidant activity was observed in baby spinach grown under red and yellow shade nets (0.01 g·kg^{-1}). Similar trend was evident with flavonoid content where baby spinach leaves grown under black nets maintained high level of flavonoids at 4, 10, and 20 °C during storage period compared with other shade nets and the control. The study control showed a better potential in retaining antioxidant activity over red and yellow shade nets. Results showed that black shade nettings have the potential to reduce water loss, decay incidents, and maintain flavonoid content and antioxidant activity followed by pearl and yellow nets.**

Recently, the demand for baby spinach (*Spinacia oleracea* L.) far exceeds the supply because of its health attributes and ease to use as leafy vegetable (Cocetta et al., 2014). Baby spinach is grown all year round in temperate regions (Rodríguez-Hidalgo et al., 2010). Baby spinach is considered to contain relatively high amounts of carotenoids (Bergquist et al., 2006), flavonoids, and phenolic acids (Fallovo et al., 2009). These biological compounds are reported to reduce chronic diseases, viz., heart-related diseases (Shashirekha et al., 2015), prostate cancer (Neuhouser, 2004), and dementia (Commenges et al., 2000).

The recent rise in global warming across the world has posed severe challenges to crop production. Among others, the challenges include increase in air temperature (AT) and intensity of solar radiation (Meena et al., 2014). Thus, more advanced and innovative practices are required to mitigate such challenges with less energy cost. The most efficient method will possibly be the use of shade nets because they can modify environmental conditions. Photoselective nets have been used to improve crop productivity and protect against pests and physical damages (Selahle et al., 2014).

Owing to adverse climate change, agronomic practices such as shade netting have been established in the past decade with the aim of filtering selective regions of the spectrum from natural radiation (Stamps, 2009). The use of colored shade nets creates favorable microclimate and provides physical protection to the crops (Oren-Shamir et al., 2001; Shahak et al., 2008), thereby improving the quality of vegetables.

The acceptability of processed produce by consumers relies on two fundamental quality parameters, visual appearance and texture (Toivonen and Brummell, 2008). Cultural practices such as sowing and harvest time (Bergquist et al., 2007a), irrigation (Pellegrini et al., 2003), mineral nutrition (Zikalala et al., 2016), industrial processing (Hodges and Toivonen, 2008), and postharvest storage temperature and duration (Mudau et al., 2015) have been reported to improve nutritional quality of baby spinach. Meena et al. (2014) demonstrated that light intensity, incoming radiation, and canopy temperature were significantly reduced under protected shade nets. Bergquist et al. (2007a) reported that shade nettings reduced flavonoid concentration in baby spinach ranging between 15% and 24%. In another study conducted by Bergquist et al. (2007b), baby spinach grown under shade nets showed significantly higher concentrations of total carotenoids and total chlorophylls. The authors further observed that the effect of shade nettings also significantly decreased ascorbic acid concentration. Data that describe the effect of a wide range of photoselective colored nets on the postharvest quality of baby spinach are lacking in South Africa. Therefore, the aim of this study was to investigate the effect of photoselective nettings on growth, productivity, and postharvest quality attributes of baby spinach.

Materials and Methods

Experimental sites. The experiments were conducted at Tshwane University of Technology Experimental Farm located in Bon Accord, north of Pretoria, at lat. 50°37′S, long. 28°12′E′′, and altitude 1173 m above sea level; the site received low summer rainfall (160.6 mm) and high temperature (>35 °C) for two consecutive seasons, viz., 2013 and 2014, respectively.

Plant material. Ohio baby spinach cultivar [Hygrotech (Pty) Ltd., Pretoria, South Africa] were grown from seeds on 10 Dec. 2013, and the trial was repeated on 18 Nov. 2014 within a glasshouse with the temperature controlled using a pad and fan system. Seedlings were thinned after 12 d of sowing. Growing bags with seedlings were spaced at 30 × 30 mm and subjected to photoselective colored shade nettings [ChromatiNet; Polysac Plastics Industries (Pty) Ltd., Israel] and open field (exposure to full sunlight).

Treatment and experimental design details. Five treatments consisted of open field (control), black, pearl, yellow, and red net arranged in a randomized complete block design with three blocks assigned to each of the five treatments. All trials were repeated twice. The shading intensities of pearl, yellow, and red nets were 40%, whereas that of black net was 25%. Plants were fertilized with a balanced nutrient solution as described by Nemadodzi et al. (2016). All plants were drip-irrigated. Drip irrigation was controlled by a computerized irrigation system, and it was two drippers per bag. One dripper had a discharge rate of 23.4 mL·min^{-1} (per bag it was 46.8 mL·min^{-1}) at 3 h intervals, five times a day.

After 35 d of sowing, all plants were harvested, and leaves were washed using running water, dried using ventilation fan, and packed in perforated 150 mm^2 plastic

Received for publication 23 Feb. 2017. Accepted for publication 4 Apr. 2017.

This work was financially supported by the Gauteng Department of Agriculture and Rural Development (GDARD; GUN no. 359000).

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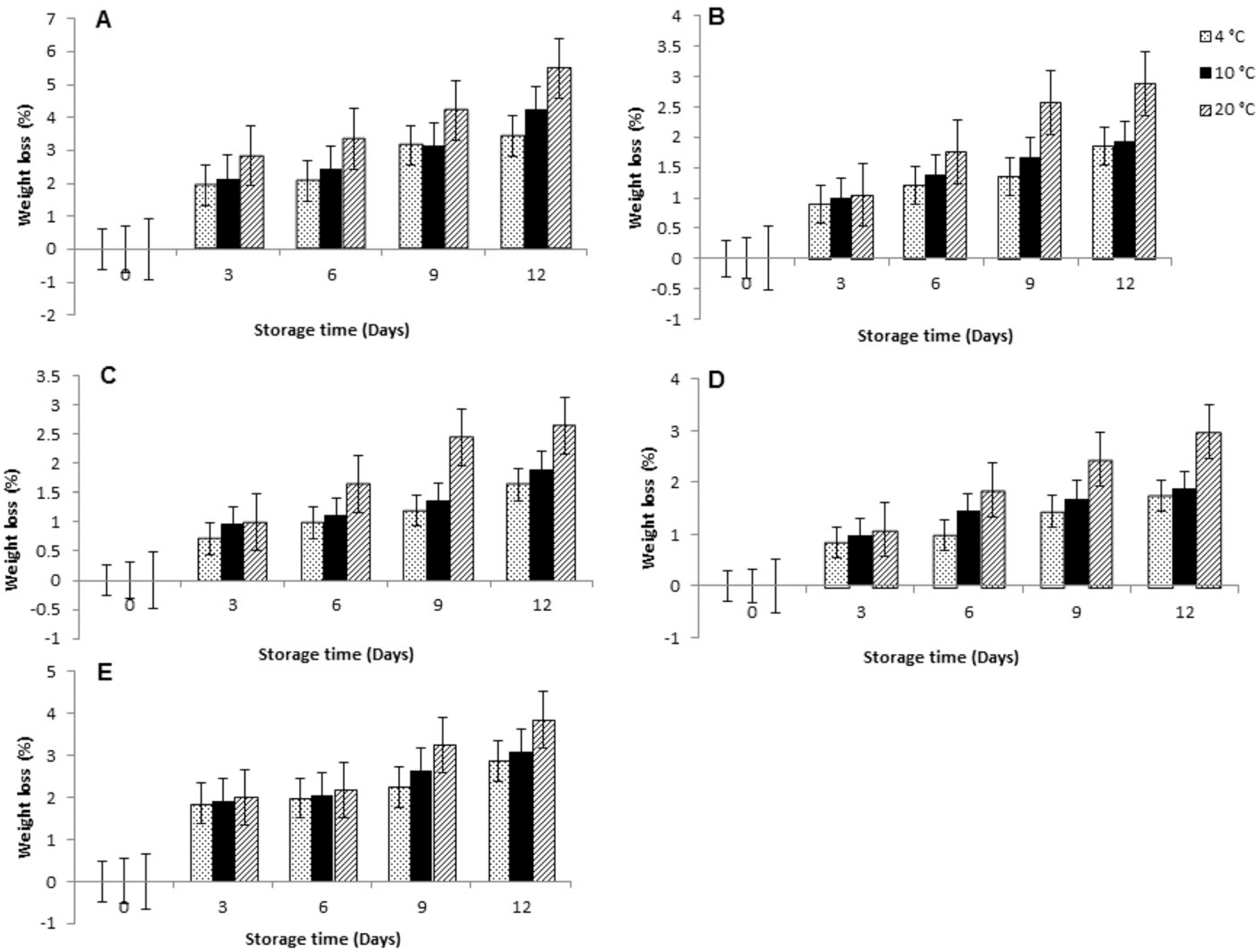

Fig. 1. Percentage cumulative weight loss of baby spinach grown under (**A**) black, (**B**) red, (**C**) pearl, (**D**) yellow, and (**E**) open field and stored at 4, 10 and 20 °C for 12 d. Data points indicate mean weight loss standard error.

Table 1. Effect of photoselective nets on petiole and leaf parameters of baby spinach.

Nets	Petiole length (cm)	Leaf width (cm)	Leaf length (cm)	Large[z] leaves (%)	Leaf chlorophyll (SPAD units)
Black	3.5 b	3.3 b	6.2 b	5	66.32 a
Red	4.3 a	3.6 b	7.5 a	6	56.38 b
Pearl	4.1 a	3.5 b	7.0 a	6	53.67 b
Yellow	4.9 a	4.2 a	7.9 a	10	56.53 b
No net	3.6 b	3.1 b	5.5 c	0	50.45 b

Means in the same column followed by different letters indicate significance ($P < 0.05$) using Duncan's multiple range test. Leaves and petioles were measured after 35 d of sowing before termination of trial.
[z]Leaves having petiole length equal to, or longer than 10 cm.

Table 2. *PAR*, AT, and RH measurements under different photoselective nets and open-field conditions.

Nets	*PAR* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	AT (°C)	RH (%)
Black	1,224.33 ± 75.06 b[z]	32.7 ± 6.5 b	50.16 ± 14.1 a
Red	768.91 ± 10.52 d	33.9 ± 8.4 b	39.1 ± 11.3 b
Pearl	855.16 ± 64.85 c	33.4 ± 7.9 b	35.9 ± 9.6 b
Yellow	745.98 ± 69.23 d	38.1 ± 8.5 a	33.6 ± 8.3 b
Open field (control)	1,561.00 ± 80.19 a	40.0 ± 12.6 a	25.0 ± 5.5 c

PAR = photosynthetically active radiation; AT = air temperature; RH = relative humidity.
Means in the same column followed different letters indicate significance ($P < 0.05$) using Duncan's multiple range test.
[z]Values are means followed by standard deviation.

containers. The baby spinach perforated containers were further stored at 4, 10, or 20 °C for 0, 2, 4, 6, 8, or 12 d, and the samples were freeze-dried.

Data collected. The following data were collected: weight loss, morphological quality (leaf length, petiole length, and leaf width), leaf chlorophyll, microenvironment analysis, total flavonoids, antioxidant activity, and sensory quality (overall acceptance, flavor/taste, and odor). Descriptions on the data collected are provided below.

Weight loss. Baby spinach leaves were weighed using a digital electronic balance [Model MK-500C, DENVER Instrument (±0.001 g); Sigma-Aldrich, St. Louis, MO] at the beginning and end of each storage period. Data were expressed as percentage weight loss.

Morphological quality and leaf chlorophyll. Parameters recorded during growing stages were petiole length, leaf length, leaf width, and leaf chlorophyll. Fifteen random plants per shade net or open field were measured after 35 d of sowing. Plant petiole length, leaf length, and leaf width were measured in centimeters (cm) using a Vernier caliper (Mitutoyo Corporation, Japan). Leaf chlorophyll content was measured nondestructively using a SPAD 502 chlorophyll meter (Konica Minolta Co. Ltd., Japan).

Microenvironment analysis. The measurements of light spectra and microclimate parameters under nets were determined using the method described by

Shahak et al. (2004). Photosynthetically active radiation (*PAR*) outside and under the nets were measured with a model LP-80 Ceptometer AccuPAR (Decagon Devices Ltd., Pullman, WA). Radiation, AT, and relative humidity (RH) were measured using Tinytag T/RH data loggers (Gemini Data Loggers Ltd., Chichester, UK) placed above the plant canopy at 1.5 m from the ground, and they were protected against direct solar radiation and rain.

Total flavonoid assay. The calorimetric protocol of Yoo et al. (2008) and slightly modified by Mudau et al. (2015) was followed to determine the total flavonoids of the baby spinach extracts.

Antioxidant activity assay. The Trolox equivalent antioxidant capacity assay described by Awika and Rooney (2004) and slightly modified by Mudau et al. (2015) was used to determine antioxidant activity (by free radical scavenging) of the baby spinach extracts.

Sensory analysis (overall appearance, flavor/taste, and odor). Product quality was evaluated in fresh harvested material and after 3, 6, 9, and 12 d of storage. An interval of a few days was opted to be able to depict any changes immediately. Sensory attributes were scored based on the methods described by Zhan et al. (2012). In this method, the overall acceptance, flavor/taste, and odor evaluation were carried out by 10 trained panelists between 23 and 45 years of age (70% females and 30% males). The panelists evaluated the overall acceptance of the fresh product at each storage interval. In an assessment of postharvest decay condition, samples were individually scored in accordance to a structured scale (from 1 to 5), where 5 = excellent, 4 = good (some leaves were slightly yellow or decayed), 3 = fair (with acceptable marketability), 2 = poor (dominated by yellow or decayed leaves), and 1 = extremely poor (inedible). With regard to odor, a scale of 1 to 5 was applied (where 5 = excellent, 4 = slightly off-odor, 3 = moderate off-odor, 2 = strong off-odor, and 1 = intolerable off-odor). In determining flavor, a scale of 5 = sweet, 4 = mild sweet, 3 = slightly sweet, 2 = bitter/sour, and 1 = poor taste (not tasted avoiding risks of microorganisms contamination) was used. A score below 3 for any of these sensory attributes was considered to be of unacceptable marketability.

Statistical analysis. Data were subjected to analysis of variance using IBM SPSS statistics version 23.0. The Pearson's correlation was performed to determine the relationship between the treatments and variables. The Wilcoxon–Mann–Whitney test was used for sensory analysis in comparison of treatments applied during postharvest. The significant difference was considered when $P < 0.05$.

Results

Weight loss. Weight loss observed during the storage period significantly varied with respect to different shade nets. However, the loss never exceeded 5.5% in all treatments (Fig. 1A–E). At day 3 of storage, spinach leaves grown under the pearl net and stored at 4 °C resulted in the least weight loss (0.72%). The plants grown under the black net had significant weight loss reaching 2.85% when stored at 20 °C. After 9 d of storage, plants grown under the black net had significantly more weight loss (4.23%) than other plants grown under nets and in an open field, whereas plants grown under the pearl net shade had the lowest weight loss of 1.2%. Generally, plants subjected to the black net across all storage temperature were found to have the highest weight loss than all

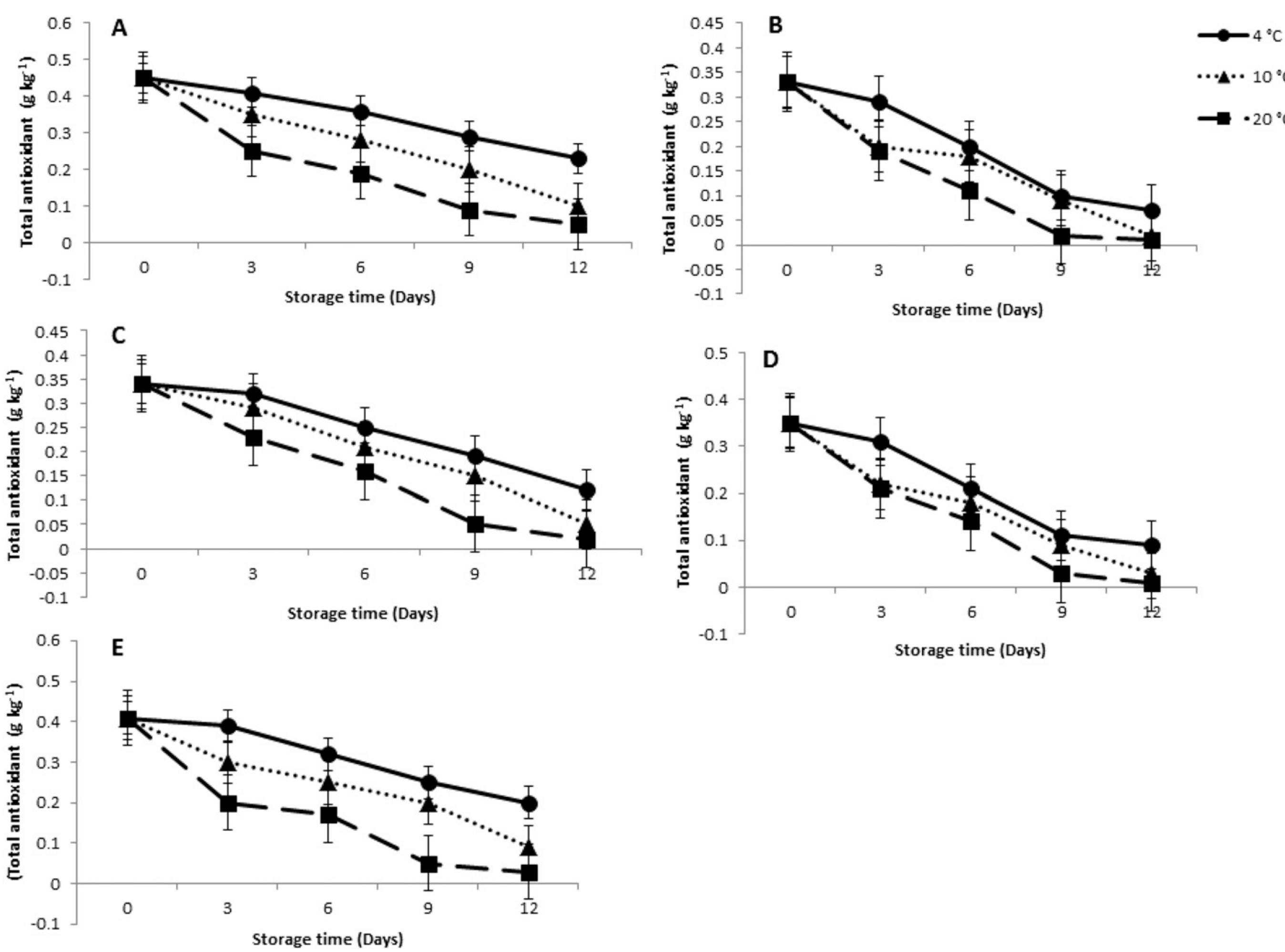

Fig. 2. Total antioxidant activity in the baby spinach leaves grown under (**A**) black, (**B**) red, (**C**) pearl, (**D**) yellow, and (**E**) open field and stored at 4, 10 and 20 °C for 12 d. Data points indicate mean total antioxidant content standard error.

treatments. Overall, the least weight loss (0.72%) was observed in plants grown under the pearl net and stored at 4 °C, whereas the highest weight loss (5.5%) was observed in plants grown under the black net and stored at 20 °C. No significant differences were observed in plants grown under red, pearl, and yellow nets at 4, 10, and 20 °C, respectively. However, plants grown under the black net showed a significant weight loss ($P < 0.05$) compared with plants grown under red, pearl, and yellow nets (Fig. 1A–E).

Morphological quality and leaf chlorophyll. Table 1 shows that baby spinach grown under photoselective nets except for the black net had significantly longer leaf petiole than those grown in an open-field condition. Pearl, red, and yellow nets exhibited longer leaf petiole than the black net.

Red, pearl, and yellow nets exhibited significant difference ($P > 0.05$) in petiole length, leaf length, and large leaves. Plants grown in the open field produced the least leaf width (3.1 cm) and leaf length (5.5 cm). High leaf chlorophyll was observed in the black net (66.32 $\mu mol \cdot m^{-2}$) followed by red (56.38 $\mu mol \cdot m^{-2}$) and yellow (56.53 $\mu mol \cdot m^{-2}$) nets (Table 1).

Microenvironment analysis. Baby spinach plants grown under field conditions were subjected to higher *PAR* of 1561.00 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than plants grown under colored shade nets (Table 2). The open field was followed by the black net with 1224.33 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Pearl net had higher *PAR* than both red and yellow nets. However, the *PAR* was least pronounced in the yellow net with a value of 745.98 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The highest AT was recorded in the open field at 40 °C, whereas the lowest AT was observed in the black shade net with 32 °C. Both red and yellow nets had AT of 33.9 °C and 38.1 °C, respectively. The lowest RH was observed in the open field (25.0%), whereas the highest RH was observed under the black net (50.2%). The red net had a RH of 39.1% followed by the pearl net (35.9%) and yellow net (33.6%) (Table 2).

Total antioxidant activity. Shade nets in combination with storage temperature affected the total antioxidant activity (TAA) at harvest and during postharvest storage (Fig. 2). At harvest, TAA was found to be higher in spinach grown under the black net (0.45 $g \cdot kg^{-1}$), whereas the least activity was pronounced in baby spinach grown under the red net with a TAA content of 0.33 $g \cdot kg^{-1}$ (Fig. 2A and B). Baby spinach grown in the open field at harvest had 0.41 $g \cdot kg^{-1}$ TAA which was higher than the TAA found in spinach grown under the yellow net (0.35 $g \cdot kg^{-1}$), pearl net (0.34 $g \cdot kg^{-1}$), and red net (0.33 $g \cdot kg^{-1}$; Fig. 4B–E). During the storage period, samples stored at 4 °C showed much better capacity of retaining higher TAA than other samples stored at 10 °C and 20 °C irrespective of the growing conditions. At 6 d of storage, the highest TAA content was observed in baby spinach grown under the black net (0.36 $g \cdot kg^{-1}$) followed by baby spinach grown in the open field (0.32 $g \cdot kg^{-1}$) when stored at 4 °C. Baby spinach grown under the black net and stored at 10 °C showed a higher TAA content (0.28 $g \cdot kg^{-1}$) than those grown under the pearl net (0.25 $g \cdot kg^{-1}$), yellow net (0.21 $g \cdot kg^{-1}$), and red net (0.20 $g \cdot kg^{-1}$) when stored at 4 °C. Among the

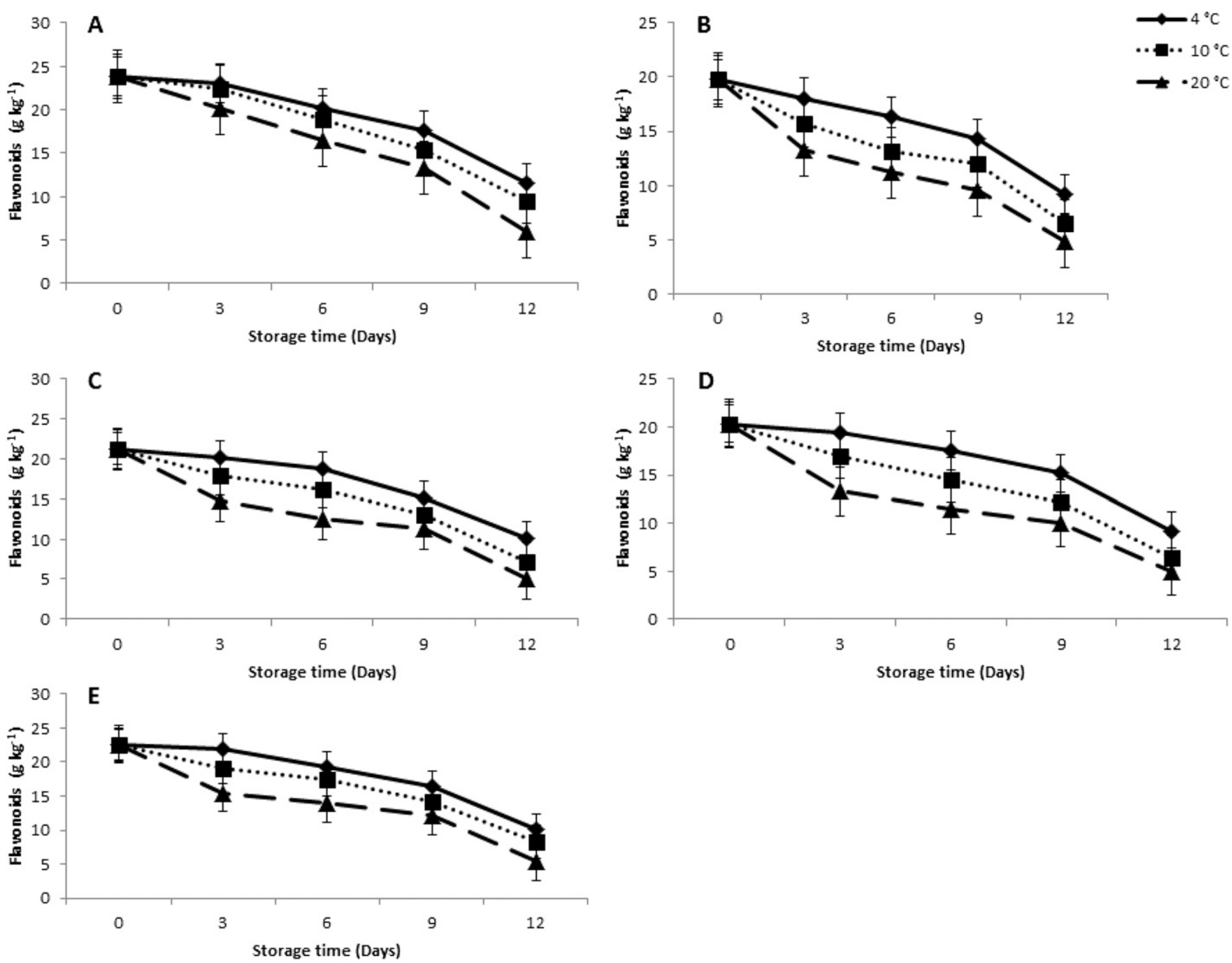

Fig. 3. Flavonoids in the baby spinach leaves grown under (**A**) black, (**B**) red, (**C**) pearl, (**D**) yellow, and (**E**) open field and stored at 4, 10 and 20 °C for 12 d. Data points indicate mean flavonoids standard error.

shade nets, spinach grown under the black net showed significantly higher ($P < 0.05$) flavonoids than the other nets when stored in different temperatures as the storage duration progressed. However, no significant difference ($P > 0.05$) was observed in the TAA content of baby spinach grown under pearl, red, and yellow shade nettings during storage duration (Fig. 2A–E).

Total flavonoids content. The effect of shade nets and storage temperature on flavonoids showed a similar trend to that observed in TAA. At harvest, spinach grown under the black net had higher flavonoid content (23.85 $g \cdot kg^{-1}$) than those grown in the open field (22.55 $g \cdot kg^{-1}$) and under the pearl net (21.25 $g \cdot kg^{-1}$), yellow net (20.32 $g \cdot kg^{-1}$), and red net [19.74 $g \cdot kg^{-1}$ (Fig. 3A–E)]. There was no consistent declining rate between the shade nets and flavonoid content during storage duration. Higher temperature (20 °C) led to great loss in flavonoid content as storage duration progressed. After 6 d of storage, spinach grown under the black net and stored at 4 °C showed higher flavonoid content than spinach grown under other nets and in the open field when stored at 4, 10, and 20 °C. Baby spinach grown under the black net had significantly higher ($P < 0.05$) flavonoid content than those grown under red, pearl, and yellow nets when stored in all three different temperatures. Baby spinach grown under the red, pearl, and yellow nets did not show significant differences in flavonoids when stored at 4, 10, and 20 °C. After 12 d of storage, the baby spinach grown under the black net had better capacity to retain the flavonoid content than those grown under other nets or without net. After 12 d of storage, the highest content of flavonoid was observed in spinach stored at 4 °C when grown under the black net followed by those grown in the open field (10.01 $g \cdot kg^{-1}$) and pearl net (10.10 $g \cdot kg^{-1}$). At the end of trial, the lowest flavonoid content was observed in spinach stored at 20 °C, when grown under the yellow net (4.95 $g \cdot kg^{-1}$) and red net (4.85 $g \cdot kg^{-1}$) (Fig. 3A–E).

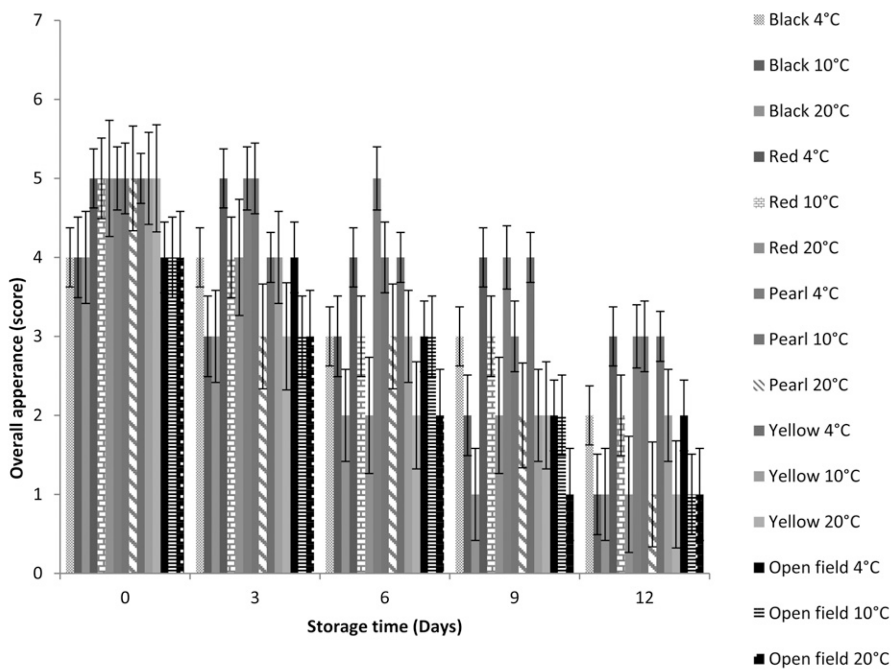

Fig. 4. Influence of photoselective nets on overall appearance in baby spinach leaves stored at 4, 10 and 20 °C for 12 d. Data points indicate mean score standard error.

Overall appearance. Results in Fig. 4 show that the quality of the baby spinach grown under black and pearl nets was greatly acceptable (using visual appearance quality) than those of that were grown under red and yellow nets. Baby spinach grown in the open field exhibited poor visual appearance after 6 d of storage.

Flavor and odor. Black, pearl, and yellow nets maintained better flavor of baby spinach leaves than those grown in the open field at 4 and 10 °C for 12 d (Fig. 5A). Baby spinach grown in the open field and red shade nettings achieved slightly off-odor state when stored at 4 °C for 12 d (Fig. 5B). Both black and yellow shade nettings maintained moderate off-odor at 4 °C for 12 d. Strong off-odor was noted after 12 d of storage at 4 °C in baby spinach leaves grown under the pearl shade netting (Figs. 4 and 5A and B).

Discussion

There are limited research reports on baby spinach grown in a wide range of colored shade nets. The current findings showed reduced weight loss and significant decay in spinach grown under the pearl net. The improved vegetative growth is most pronounced

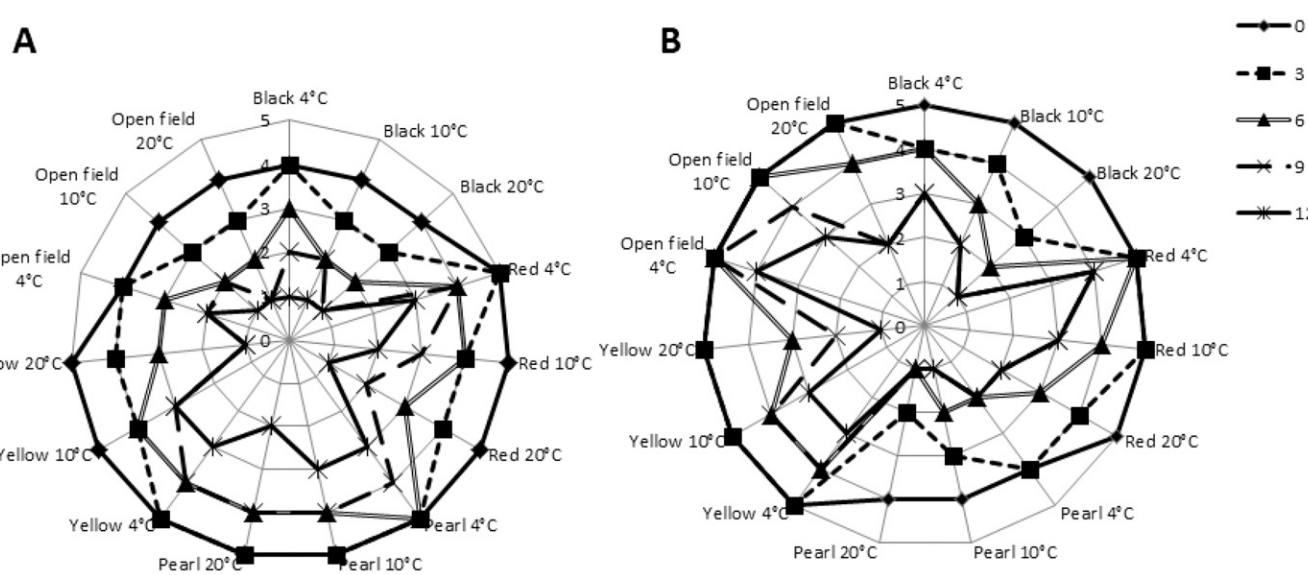

Fig. 5. Influence of photoselective nets on the (**A**) flavor and (**B**) odor in baby spinach leaves stored at 4, 10, and 20 °C for 12 d.

in plants grown under shade nets in comparison with plants grown in the open field. Iglesias and Alegre (2006) reported that the increase in RH, lower maximum temperature, and lower wind speed within the protected environment reduces incidence of water loss and subsequent reduction in decay. These extreme conditions let unprotected plants being exposed to high heat stress, particularly baby spinach which requires cool conditions for better growth and development. Oren-Shamir et al. (2001) reported that black and red nets promote elongation during growth and development processes.

Shade nets are mainly deployed over crops to lessen heat stress (Shahak et al., 2004). Photoselective nets are regarded as nets which prevent excess radiation and improve the soil moisture levels that promote proper plant growth. This improves the productivity (yield) of the plant and, arguably, retains the best quality of leaves during postharvest storage. The reduction of radiation and AT is responsible for a great deal in regulating the photosynthetic capacity of leaves and, consequently, a reduced light saturated photosynthetic rate unlike in the open field (Medany et al., 2008). Ilić et al. (2015) observed similar findings when plants under the black net had higher chlorophyll content than other selected photoselective nettings. According to Shahak et al. (2008), lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) had significantly increased production when subjected to the red and pearl nets over those grown under aluminet, blue, or black nets.

Phenolic compounds are major constituents of antioxidant properties of fresh produce (Gil et al., 2002). With the lack of data that describe the physiological responses of baby leaf spinach on phytochemicals on pearl as photoselective net, the present findings exhibited an improved antioxidant activity compared with plants grown under a pearl net.

It is known that the response of individual crop species to harmful ultraviolet-B radiation can differ considerably in the process of flavonoid synthesis (Harborne and Williams, 2000). Quercetin and its glycosides are extensively evaluated flavonoids for their biological activities (Proteggente et al., 2002). This is evident in our findings that there is similar response of antioxidant activity and flavonoids in baby spinach. Conversely, Bergquist et al. (2007a) reported that there was an increase in the flavonoids in spinach grown under shade during storage period. This increase was attributed to induced stress caused by shade and, as a result, increased flavonoid concentration.

In conclusion, the results of this study demonstrated that the black net reduces water loss and decay incidents, and maintains the content of flavonoid and antioxidant activity followed by pearl and yellow nets, respectively. The results also suggested that photoselective shade nets technology can be recommended when growing baby spinach particularly under black, pearl, and yellow nets when baby spinach leaves are stored at 4 °C during storage.

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