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# ETHANOL ADDED TO WASHING WATER DELAYS YELLOWING IN *Spinacia oleracea* L. cv. 'Matador'

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## ABSTRACT

The primary quality concern for spinach and other green vegetables during post-harvest handling is preserving the green color, specifically by delaying the yellowing caused by chlorophyll loss. The current study, therefore, aimed to investigate the effect of ethanol added to washing water in low concentrations on yellowing, chlorophyll loss, and the storage quality of spinach. For this purpose, ethanol was added to tap water at 0 µL $L^{-1}$ (control), 200 µL $L^{-1}$, 400 µL $L^{-1}$, and 800 µL $L^{-1}$, and after pre-washing, the spinach leaves were dipped in these solutions at a temperature of 16–18°C for five minutes. The spinach was stored at 4 ±1°C and at 90–95% RH for 21 days after being drained, dried, and packed, and the quality parameters were recorded at seven-day intervals. As a result of this study, the decline in chlorophyll losses was obtained especially by the application of the 400 µL $L^{-1}$ ethanol treatment after the first 14 days of storage, and this result was positively correlated with both the color values $L^*$, $a^*$, $b^*$, hue, yellowness index (YI), total color difference (ΔE), and the chlorophyll SPAD (soil-plant analysis development) values. Consequently, adding 400 µL $L^{-1}$ of ethanol to the washing water was the most effective in delaying yellowing and chlorophyll loss in spinach. However, this effect declined with increasing solution concentrations and was accompanied by weight loss.

**Keywords:** chlorophyll loss, green vegetables, storage quality, leaf greenness index (SPAD), spinach

## INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a popular green leafy vegetable with rich nutrient contents. It comprises significant content of flavonoids, folates, carotenoids, polyphenols, ascorbic acid (vitamin C), β-carotene, various minerals (iron, potassium, and magnesium), and antioxidants [Koh et al. 2012, Murcia et al. 2020]. Thanks to these nutrients, spinach decreases the risks of cardiovascular diseases, diabetes, and strokes and has positive effects such as lipid-lowering, anti-inflammatory characteristics, and anti-cancer properties [Shashirekha et al. 2015, Roberts and Moreau 2016]. The increase in the awareness of consumer health resulted in an increased consumption and production of spinach [Morelock and Correll 2008].

The most important indicator of quality loss of spinach and other green vegetables during the post-harvest process is yellowing from chlorophyll loss [Zhu et al. 2017, Chakraborty and Chattopadhyay 2018]. Maintaining the green color of spinach at the same time means protecting its commercial value [Kaur et al. 2011]. The dark storage of spinach improves visual quality criteria such as freshness (brightness-crispness), a delay in decay, and the retention of tissue integrity [Koike et al. 2011, Grozeff et al. 2013],

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while causing increasing yellowing [Hodges and Toivonen 200].

Ethanol is an anaerobic metabolite used for different purposes in horticultural crops [Pesis 2005], both in liquid [Lin et al. 2020, Dorostkar and Moradinezhad 2022] and vapor [Wang et al. 2011, Thewes et al. 2021] forms. Ethanol is known as a broad-spectrum antifungal agent for pathogenic infection of fruit and vegetables because of its lethal effect on the mitochondrial membranes of fungus spores [Sahoo et al. 2021]. Nevertheless, ethanol treatments are used for different purposes, such as delaying softening via retarding the deterioration of cell-wall polysaccharides in blueberries [Ji et al. 2021], retarding post-harvest physiological disorders in cassavas [Liu et al. 2019a], preventing decay during the storage of grapes [Candir et al. 2012, Romero et al. 2021], increasing the sensory quality of cherry tomatoes [Liu et al. 2019b], preventing ethylene production and delaying decay in bananas [de França et al. 2019], delaying softening in blueberries [Ji et al. 2021], inhibiting internal ethylene biosynthesis in oriental sweet melons and tomato [Jin et al. 2013, Suzuki and Nagata 2019], preventing ripening of apples at room temperature [Thewes et al. 2021], controlling browning in fresh-cut lotus roots and apples [Yan et al. 2017, Xu et al. 2023] and preventing withering in fresh-cut Jerusalem artichokes [Wang et al. 2014].

Previous studies showed that ethanol treatments delay yellowing due to the retardation of the chlorophyll breakdown in broccoli [Fukasawa et al. 2010, Xu et al. 2012] and in lime fruit [Opio et al. 2015]. However, no study was found regarding the use of ethanol in leafy vegetables. In this context, the study aimed to investigate the effect of ethanol added in low concentrations to washing water on yellowing, chlorophyll loss, and the other quality parameters of spinach.

## MATERIAL AND METHODS

The spinach leaves (*Spinacia oleracea* L cv. 'Matador') used in the experiment were produced in the Sepetçiler village in İzmit in the Kocaeli province, Turkey. A winter spinach cultivar, 'Matador', was used in this study because it is the most preferred cultivar by producers in Turkey and suits the preferences of the consumers. The plant material was produced under an integrated agriculture regime in a single production region, and the experiment was conducted in 2021. Spinach harvested during commercial harvest time (12 November) was transported to the laboratory, washed with tap water, and then sorted and dried. Thereafter, the spinach leaves were dipped for five minutes into the different doses of ethanol solutions, including 0 µL $L^{-1}$ (C/control), 200 µL $L^{-1}$ (L-200), 400 µL $L^{-1}$ (L-400), and 800 µL $L^{-1}$ (L-800). The temperatures of the solutions were in the protected range of 16–18°C during the treatments. After dipping, the spinach leaves were drained and then dried on rough filter paper, placed into the polystyrene foam dishes weighing 200 ±10 g and wrapped with stretch film. The packaged spinach leaves were stored in a cold room with a temperature of 4 ±1°C and at 90–95% RH. The measurements and analyses, as follows, were done on the initial day of the seven-day intervals during storage. Analyses were performed in three replicates, and each dish was considered as one replicate.

**Color measurement.** The color of the spinach leaves was measured using a Minolta CR 400 chromameter with a D65 lamp (Minolta Co, Osaka, Japan). The measurement was taken at three points on each leaf, and five leaves were used for each replicate. The CIELAB color coordinate system ($L^*a^*b^*$) was used to refer to the leaf color. The $L^*$ represent the brightness of the color (0 = black, 100 = white), when it increases towards 100, the brightness increases. Value $a^*$ varies between –60 and +60, (–) indicates green and (+) indicates yellowness. The hue angle (H°) values of 0°, 90°, 180° and 270° mean red, yellow, green, and blue, respectively [Konica Minolta, 2023]. The H°, the total color difference (ΔE), and the yellowness index (YI) [Hirschler, 2012] were calculated using the formulas given below:

$$H^o = 90 + tan^{-1}(\frac{b^*}{a^*})$$

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}$$

$$YI_{FC} = 142.86\ \frac{b^*}{L^*}$$

**Chlorophyll SPAD value**. Chlorophyll SPAD content was measured using a SPAD-502 Plus Konica Minolta chlorophyll meter (Minolta Co, Osaka, Japan) at

three points of each leaf, and five leaves were used for each replicate (n = 15).

**Chlorophyll content.** To calculate the leaf chlorophyll content, a 500 mg sample was extracted three times in 5 mL 80% acetone and filtered, and the final volume was equalized in all the samples. The absorbance values of the samples were read at 645 and 663 nm wavelengths using the spectrophotometer (UV-1280, Shimadzu, Japan) [Ni et al. 2009]. The chlorophyll *a*, *b*, and total chlorophyll content values were calculated using the formulas as follows:

$$Kl_a(mg\ g^{-1}) = \frac{(12.7 \times A_{663}) - (2.69 \times A_{645})}{W} \times V$$

$$Kl_b(mg\ g^{-1}) = \frac{(22.9 \times A_{645}) - (4.86 \times A_{663})}{W} \times V$$

$$Kl_{a+b}(mg\ g^{-1}) = \frac{(8.02 \times A_{663}) + (20.20 \times A_{645})}{W} \times V$$

where: $Kl_a$: chlorophyll *a* (mg $g^{-1}$), $Kl_b$: chlorophyll *b* (mg $g^{-1}$), $Kl_{a+b}$: total chlorophyll (mg $g^{-1}$), $A_{645}$: absorbance at 645 nm, $A_{663}$: absorbance at 663 mm, v: volume of the extract (mL), W: fresh leaf weight (mg).

**Total soluble solids (TSS).** Spinach was squeezed with a hand press to determine the TSS content (%). The solid phase was separated from the liquid phase by filtering with fast-flow filter paper, and the liquid phase was measured using an Atago Pal-3 (Atago Co., Ltd., Tokyo, Japan) digital refractometer.

**Weight loss.** Weight loss was calculated using the following formula: WL (%) = (initial weight – final weight) × 100 / initial weight.

**Sugar analysis.** Three grams of fresh samples and 15 mL of HPLC grade water were placed in a beaker and then homogenized using a Waggenhauser D-500 ultra turrax homogenizer (Waggenhauser Berlin, Germany) for three minutes at a low speed. After homogenization, the samples were first filtered on rough filter paper, followed by a nylon 66 syringe filter, and were then injected into the HPLC.

HPLC conditions: Agilent, HP 1210, Zorbax carbohydrate column (4.6 mm ID × 150 mm, 5 µm), mobile phase: acetonitrile ($CH_3CN$): water ($H_2O$) (75:25), flow speed, 1.4 mL $min^{-1}$, column temperature, 30°C, detector: HP110 RID, detector temperature, 30°C, injection volume, 20 µL. The standard curve, which was created by five different concentrations of the stock solutions, was used for glucose, fructose, and sucrose quantity analysis.

**Experimental design.** The study was established according to a completely randomized plot pattern using a factorial design with three replicates of one packed spinach for each repetition. Ethanol treatments were evaluated as factor I, and periods of analyses (initial, 7, 14, and 21 days) were factor II. The SPSS 16 software program was used for the variance analyses of the data, and measurements and analyses to determine significant differences were compared using the Tukey comparison test within 5% error limits.

## RESULTS

### Color evaluation

The color of spinach was evaluated using five different parameters (Tab. 1). During storage, *a** color values decreased quickly in the control and L-200 treatment, but the decline in the L-200 group was slower than in the control group. There were insignificant differences in the *a** values of the spinach in the L-400 and L-800 groups, although the *a** values of the L-400 group were higher than the values of the L-800 group during all analyzed periods. The increase occurred in the *b** value depending on the process of spinach yellowing during storage. This increase was the highest in the control group (from 17.03 to 24.52), while it was the lowest in the L-400 group (from 17.03 to 18.73). The hue angle was 127.11 at harvest and declined during storage. The highest decrease was found in the control group (120.57 on day 21), while the lowest decrease was found in the L-400 group (123.55 on day 21). The H° was retained by all ethanol treatments until the day 7, and then this effect declined. Yellowness indices generally decreased in all ethanol treatments, but this effect in the L-200 and L-800 groups was lost towards the end of storage. However, in the L-400 group, the YI stayed at almost the same level as at harvest during the 21 days of storage. As seen in Table 1, significant changes in the ΔE value were noted. This parameter reached the highest level of 10.0 in the control group at the end of the storage period, while it reached a value of 4.0 in the L-400 group, which was 60% lower than the value obtained in the control group.

**Table 1.** Effects of different ethanol treatments on color parameters of *Spinacia oleracea* L cv. 'Matador' during storage

| Values of color parameters | | | | | | |
|---|---|---|---|---|---|---|
| Color parameters | Ethanol treatment | Storage times (days) | | | | Means for ethanol treatment[y] |
| | | 0 | 7 | 14 | 21 | |
| Lightness (L*) | C | 34.69 ±1.08 | 37.41 ±0.16 | 37.87 ±1.09 | 41.10 ±1.22 | 37.77 a |
| | L-200 | 34.69 ±1.08 | 36.56 ±1.90 | 37.34 ±0.34 | 39.64 ±1.50 | 37.06 ab |
| | L-400 | 34.69 ±1.08 | 35.45 ±1.52 | 36.34 ±0.99 | 38.15 ±0.91 | 36.16 b |
| | L-800 | 34.69 ±1.08 | 37.10 ±0.31 | 36.45 ±0.28 | 38.90 ±2.01 | 36.79 ab |
| | Means for storage time[y] | 34.69 d | 36.63 b | 36.99 b | 39.45 a | |
| | Significance[z] | Storage time$^{*}$, Ethanol treatment$^{**}$, Storage time × ethanol treatment$^{ns}$ | | | | |
| Color a* | C | –12.84 ±0.79 | –13.52 ±0.88 | –12.96 ±0.86 | –14.48 ±0.10 | –13.45 c |
| | L-200 | –12.84 ±0.79 | –12.63 ±1.12 | –13.03 ±0.22 | –13.69 ±0.68 | –13.05 bc |
| | L-400 | –12.84 ±0.79 | –11.53 ±0.54 | –12.20 ±0.88 | –12.42 ±0.47 | –12.25 a |
| | L-800 | –12.84 ±0.79 | –12.66 ±0.54 | –12.59 ±0.35 | –12.99 ±0.15 | –12.77 ab |
| | Means for storage time[y] | –12.84 ab | –12.59 a | –12.80 a | –13.40 b | |
| | Significance | Storage time$^{*}$, Ethanol treatment$^{**}$, Storage time × ethanol treatment$^{ns}$ | | | | |
| Color b* | C | 17.03 ±1.93 | 19.91 ±2.18 | 19.30 ±2.36 | 24.52 ±0.60 | 20.19 a |
| | L-200 | 17.03 ±1.93 | 17.55 ±3.31 | 19.10 ±0.41 | 22.22 ±1.89 | 18.98 a |
| | L-400 | 17.03 ±1.93 | 15.51 ±1.77 | 18.14 ±0.82 | 18.73 ±0.72 | 17.36 b |
| | L-800 | 17.03 ±1.93 | 17.92 ±0.82 | 18.76 ±1.23 | 21.45 ±0.56 | 18.79 ab |
| | Means for storage time | 17.03 c | 17.72 bc | 18.97 b | 21.73 a | |
| | Significance | Storage time$^{***}$, Ethanol treatment$^{**}$, Storage time × ethanol treatment$^{ns}$ | | | | |
| Hue angle (H°) | C | 127.11 ±1.50 | 124.27 ±1.36 | 123.98 ±1.49 | 120.57 ±0.55 | 123.98 b |
| | L-200 | 127.11 ±1.50 | 126.00 ±2.54 | 124.30 ±0.75 | 121.69 ±1.52 | 124.77 ab |
| | L-400 | 127.11 ±1.50 | 126.75 ±1.86 | 123.90 ±0.64 | 123.55 ±0.04 | 125.33 a |
| | L-800 | 127.11 ±1.50 | 125.25 ±0.75 | 123.91 ±0.99 | 121.21 ±0.39 | 124.37 ab |
| | Means for storage time | 127.11 a | 125.56 b | 124.04 c | 121.76 d | |
| | Significance | Storage time$^{***}$, Ethanol treatment$^{*}$, Storage time × ethanol treatment$^{ns}$ | | | | |
| Yellowness indices (YI) | C | 70.05 ±6.31 | 76.04 ±8.64 | 72.66 ±6.76 | 85.29 ±2.74 | 76.01 a |
| | L-200 | 70.05 ±6.31 | 68.30 ±9.58 | 73.10 ±1.47 | 79.98 ±3.80 | 72.86 ab |
| | L-400 | 70.05 ±6.31 | 62.38 ±4.48 | 71.40 ±4.62 | 70.13 ±1.56 | 68.49 b |
| | L-800 | 70.05 ±6.31 | 69.01 ±3.10 | 73.52 ±4.94 | 78.85 ±2.08 | 72.86 ab |
| | Means for storage time | 70.05 b | 68.93 b | 72.67 b | 78.56 a | |
| | Significance | Storage time$^{***}$, Ethanol treatment$^{**}$, Storage time × ethanol treatment$^{ns}$ | | | | |
| Total color difference (ΔE) | C | 0.00 ±0.00 | 4.82 ±3.15 | 4.09 ±2.48 | 10.00 ±2.19 | 4.73 a |
| | L-200 | 0.00 ±0.00 | 4.56 ±3.11 | 3.66 ±1.97 | 7.27 ±2.18 | 3.87 ab |
| | L-400 | 0.00 ±0.00 | 3.42 ±2.37 | 2.22 ±1.50 | 4.00 ±1.56 | 2.41 b |
| | L-800 | 0.00 ±0.00 | 2.98 ±1.36 | 2.70 ±1.13 | 6.31 ±0.94 | 3.00 b |
| | Means for storage time | 0.00 c | 3.94 b | 3.11 b | 6.90 a | |
| | Significance | Storage time$^{***}$, Ethanol treatment$^{*}$, Storage time × ethanol treatment$^{ns}$ | | | | |

Values are the means ± SD (n = 15)

[z]Two-way ANOVA was used to test which factors and interactions between the factors had significant effects on the examined quality parameters

ns: no significant differences detected, *, **, *** significant at $p < 0.05$, 0.01 and 0.001, respectively

[y]Values followed by different letters are significantly different at $p = 0.05$, according to the Tukey test

### Chlorophyll analyses

The highest decrease in chlorophyll SPAD value during storage was determined in spinach of the control group (Tab. 2). While the chlorophyll SPAD value, which was 48.23 at harvest time, decreased dramatically to 41.67 in the control group at the end of storage, this was followed by spinach of the L-200, L-400 and L-800 groups (43.60, 44.70, 45.10, respectively). The decrease rate was 7.3% and 6.5% in the L-400 and L-800 groups, respectively, and the differences between these two groups were statistically significant.

Chlorophyll losses were observed in all the treatment groups during storage (Fig. 1). While the chlorophyll *a* content of spinach in the control group was 0.82 mg $g^{-1}$ at the time of harvest, 47.5% of its content was lost at the end of storage and decreased to 0.43 mg $g^{-1}$, while 39% of this loss occurred on day 7 (0.5 mg $g^{-1}$). The chlorophyll loss of the spinach in the L-200 group was close to the control group. In other words, L-200 was not successful in retarding chlorophyll loss. Despite that, chlorophyll *a* loss was slowed by both the L-400 and L-800 treatments, and the loss rate on day 7 was only 16.9% (0.68 mg $g^{-1}$) in

**Table 2.** Effects of different ethanol treatments on SPAD values of *Spinacia oleracea* L cv. 'Matador' during storage

| SPAD values | | | | | |
|---|---|---|---|---|---|
| Ethanol treatment | Storage times (days) | | | | Means for ethanol treatment[y] |
| | 0 | 7 | 14 | 21 | |
| C | 48.23 ±3.25 | 43.74 ±3.47 | 43.80 ±1.28 | 41.67 ±2.84 | 44.36 b |
| L-200 | 48.23 ±3.25 | 47.98 ±1.84 | 46.23 ±0.93 | 43.60 ±2.44 | 46.51 ab |
| L-400 | 48.23 ±3.25 | 50.33 ±5.45 | 45.87 ±3.39 | 44.70 ±3.65 | 47.28 a |
| L-800 | 48.23 ±3.25 | 50.01 ±1.04 | 46.83 ±1.95 | 45.10 ±3.65 | 47.54 a |
| Means for storage time | 48.23 a | 48.01 a | 45.54 ab | 43.77 b | |
| Significance[z] | Storage time$^{**}$, Ethanol treatment$^{*}$, Storage time × ethanol treatment$^{ns}$ | | | | |

Values are the means ± SD (n = 15)
[z]Two-way ANOVA was used to test which factors and interactions between the factors had significant effects on the examined quality parameters
ns: no significant differences detected, *, **, *** significant at $p < 0.05$, 0.01 and 0.001, respectively
[y]Values followed by different letters are significantly different at $p = 0.05$, according to the Tukey test

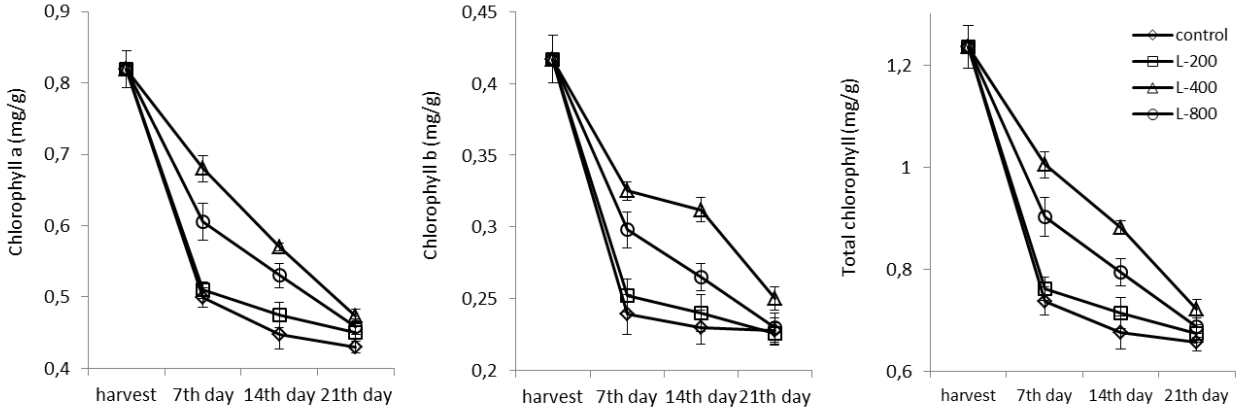


**Fig. 1.** Effects of different ethanol treatments on chlorophyll *a*, chlorophyll *b*, and total chlorophyll content of *Spinacia oleracea* L cv. 'Matador' leaves during storage. Values are the means ±SD of three replicate assays. Significance: storage time $p < 0.01$, ethanol treatment $p < 0.01$, storage time × ethanol treatment was insignificant

L-400 and 26.1% (0.61 mg $g^{-1}$) in the L-800 groups, respectively. Similar results were obtained for chlorophyll *b*. The amount of chlorophyll *b* in the control samples, which was 0.42 mg $g^{-1}$ at the time of harvest, decreased by 45.4% to 0.23 mg $g^{-1}$ at the end of storage, and 42.7% of this loss occurred on day 7 of storage. From this point, the most effective treatment was L-400, where chlorophyll *b* loss was 22% on day 7, 25.2% on day 14, and 40% at the end of storage. The L-400 treatment was also effective in decreasing total chlorophyll loss. After 7 and 14 days of storage, the total chlorophyll losses were 18.7% and 28.6%, respectively. Total chlorophyll losses were also higher in the control and L-200 groups compared to the L-400 group in both storage periods. Additionally, the L-800 application reduced total chlorophyll losses on day 7 (26.9%), but this reduction was limited compared to L-400 on day 14 (37.7%).

### Total soluble solids (TSS)

The TSS content of the spinach leaves in all treatments slightly increased during the first 14 days of storage, but this increase was not significant at the end of storage (Tab. 3). Thus, there was no significant effect of ethanol treatments on the TSS content of the spinach.

**Table 3.** TSS content of the spinach leaves treated with different doses of ethanol

| Total soluble solids (TSS) content | | | | | |
|---|---|---|---|---|---|
| | Storage times (days) | | | | Means for ethanol treatment[y] |
| Ethanol treatment | 0 | 7 | 14 | 21 | |
| C | 3.12 ±0.67 | 2.53 ±0.31 | 3.00 ±0.76 | 4.03 ±0.12 | 3.17 a |
| L-200 | 3.12 ±0.67 | 3.40 ±0.40 | 3.27 ±0.40 | 5.07 ±0.86 | 3.71 a |
| L-400 | 3.12 ±0.67 | 3.70 ±0.95 | 3.63 ±0.47 | 4.47 ±0.96 | 3.73 a |
| L-800 | 3.12 ±0.67 | 3.20 ±0.36 | 3.67 ±0.59 | 4.37 ±1.01 | 3.59 a |
| Means for storage time | 3.12 b | 3.21 b | 3.33 b | 4.48 a | |
| Significance[z] | Storage time[***], Ethanol treatment[ns], Storage time × ethanol treatment[ns] | | | | |

Values are the means ± SD (n = 3)
[z]Two way ANOVA was used to test which factors and interactions between the factors had significant effects on the examined quality parameters
ns: no significant differences detected, *, **, *** significant at $p < 0.05$, 0.01 and 0.001, respectively
[y]Values followed by different letters are significantly different at $p = 0.05$, according to the Tukey test

**Table 4.** Effects of the different doses of ethanol treatments on *Spinacia oleracea* L cv. 'Matador' weight loss during storage

| Weight loss (%) | | | | | |
|---|---|---|---|---|---|
| Ethanol treatment | Storage times (days) | | | | Means for ethanol treatment[y] |
| | 0 | 7 | 14 | 21 | |
| C | 0.00 ±0.0 | 9.04 ±0.85 | 16.74 ±1.38 | 24.70 ±1.90 | 12.62 b |
| L-200 | 0.00 ±0.0 | 8.72 ±0.30 | 16.20 ±0.63 | 23.83 ±0.83 | 12.19 b |
| L-400 | 0.00 ±0.0 | 10.07 ±0.60 | 18.43 ±1.06 | 26.52 ±1.45 | 13.75 b |
| L-800 | 0.00 ±0.0 | 11.51 ±2.53 | 20.59 ±3.50 | 28.70 ±3.33 | 15.20 a |
| Means for storage time | 0.00 d | 9.83 c | 17.87 b | 25.94 a | |
| Significance[z] | Storage time[***], Ethanol treatment[***], Storage time × ethanol treatment[ns] | | | | |

Values are the means ± SD (n = 3)
[z]Two-way ANOVA was used to test which factors and interactions between the factors had significant effects on the examined quality parameters
ns: no significant differences detected, *, **, *** significant at $p < 0.05$, 0.01 and 0.001, respectively
[y]Values followed by different letters are significantly different at $p = 0.05$, according to the Tukey test

### Weight loss (%)

It was found that increasing ethanol treatment doses produced higher weight losses in the experimental spinach (Tab. 4). The highest weight loss was measured in the L-800 group (on day 21, 28.7%), and the differences between this group and the others were significant. Even though the weight loss was higher in the L-400 group than in the control and L-200 groups, the differences among the treatments were insignificant.

### Sugar content

A different result was detected in the sugar content than in the other measurements and analyses. Namely, according to two factorial variance analyses, the interaction of the storage duration and the ethanol treatment was insignificant in the other evaluations, while it was significant in all sugar measurements ($p < 0.001$) (Tab. 5). According to the results, the sugar content, which is already low in spinach, showed small fluctuations. Nevertheless, at the end of storage, the sugar content was low in the control group at 0.38%, followed by the L-200 group (0.41%), the L-400 group (0.45%), and the L-800 group (0.53%) (Fig. 2).

## DISCUSSION

The most important desired quality parameters of green leafy vegetables are freshness and the presence of a homogeneous green color [Chakraborty and Chattopadhyay 2018]. As with other vegetables, the cause of the yellowing of spinach is its quick chlorophyll breakdown, and this situation shortens the shelf life of the vegetable [Chen et al. 2008]. Among the most crucial quality losses of spinach are yellowing and water loss, which occur mainly during storage and marketing [Yamauchi 2015]. Therefore, maintaining the green color of leafy vegetables is the most important goal. The 400 µL $L^{-1}$ ethanol solution in washing water in this study, reduced color loss, retarded yellowing and delayed chlorophyll losses of the spinach. Previous studies showed that ethanol treatments delayed chlorophyll breakdown and yellowing in broccoli [Fukasawa et al. 2010, Xu et al. 2012] and in citrus

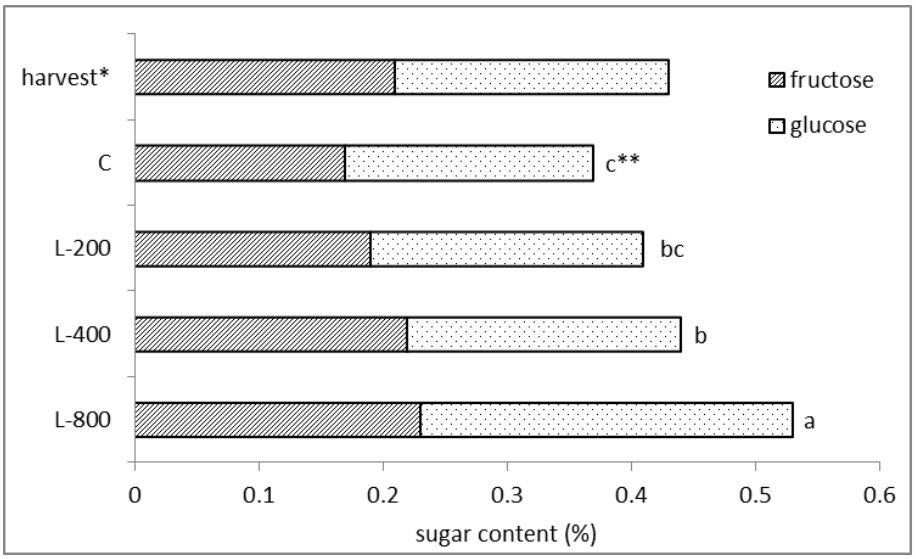


**Fig. 2.** The effect of different doses of ethanol treatments on the sugar (fructose and glucose) content of the spinach at harvest and during 21 days of storage. *In the graph, the sugar content at harvest was given with the 'harvest' tag for comparison purposes, ** in the graph, both fructose and glucose data and totals are also shown as total sugar. During twenty-one days of storage, there were significant differences within a 5% error limit among the control and three ethanol groups in terms of total sugar, as shown in the bars containing different letters

**Table 5.** Effects of different ethanol treatments on sugar contents of *Spinacia oleracea* L cv. 'Matador' during storage

| Glucose, fructose and total sugar content | | | | | | |
|---|---|---|---|---|---|---|
| Sugar content | Ethanol treatment | Storage times (days) | | | | Means for ethanol treatment[y] |
| | | 0 | 7 | 14 | 21 | |
| Fructose (%) | C | 0.21 ±0.02 | 0.22 ±0.01 a | 0.19 ±0.01 a | 0.17 ±0.01 b | 0.20 |
| | L-200 | 0.21 ±0.02 | 0.21 ±0.01 a | 0.21 ±0.02 a | 0.19 ±0.02 ab | 0.21 |
| | L-400 | 0.21 ±0.02 | 0.16 ±0.02 b | 0.18 ±0.02 a | 0.22 ±0.02 a | 0.20 |
| | L-800 | 0.21 ±0.02 | 0.14 ±0.01 b | 0.18 ±0.03 a | 0.23 ±0.04 a | 0.19 |
| | Means for storage time[y] | 0.21 a | 0.19 c | 0.19 bc | 0.20 ab | |
| | Significance[z] | Storage time$^{**}$, Ethanol treatment$^{ns}$, Storage time × ethanol treatment$^{***}$ | | | | |
| Glucose (%) | C | 0.22 ±0.02 | 0.25 ±0.03 a | 0.28 ±0.02 a | 0.20 ±0.01 b | 0.24 |
| | L-200 | 0.22 ±0.02 | 0.23 ±0.02 a | 0.25 ±0.04 a | 0.22 ±0.01 b | 0.23 |
| | L-400 | 0.22 ±0.02 | 0.22 ±0.00 ab | 0.21 ±0.01 b | 0.22 ±0.01 b | 0.22 |
| | L-800 | 0.22 ±0.02 | 0.18 ±0.01 b | 0.21 ±0.00 b | 0.30 ±0.02 a | 0.23 |
| | Means for storage time[y] | 0.22$^{ns}$ | 0.22 | 0.24 | 0.24 | |
| | Significance | Storage time$^{ns}$, Ethanol treatment$^{ns}$, Storage time × ethanol treatment$^{***}$ | | | | |
| Total sugar (%) | C | 0.44 ±0.02 | 0.48 ±0.03 a | 0.47 ±0.03 a | 0.38 ±0.02 c | 0.44 |
| | L-200 | 0.44 ±0.02 | 0.44 ±0.02 a | 0.46 ±0.05 a | 0.41 ±0.03 bc | 0.44 |
| | L-400 | 0.44 ±0.02 | 0.38 ±0.03 b | 0.39 ±0.01 b | 0.45 ±0.01 b | 0.41 |
| | L-800 | 0.44 ±0.02 | 0.33 ±0.02 c | 0.39 ±0.03 b | 0.53 ±0.03 a | 0.42 |
| | Means for storage time | 0.44 a | 0.41 b | 0.43 ab | 0.44 a | |
| | Significance | Storage time$^{*}$, Ethanol treatment$^{ns}$, Storage time × ethanol treatment$^{***}$ | | | | |

Values are the means ± SD (n = 3)
[z]Two-way ANOVA was used to test which factors and interactions between the factors had significant effects on the examined quality parameters
ns: no significant differences detected, *, **, *** significant at $p < 0.05$, 0.01 and 0.001, respectively
[y]Values followed by different letters are significantly different at $p = 0.05$, according to the Tukey test

[Noma et al. 2009, Opio et al. 2015], but no literature data was found on ethanol treatments to spinach for delaying yellowing. The leaf color of spinach, which has dark green leaves, is correlated directly with its freshness [Martínez-Sánchez et al. 2019]. As a result of reducing reactive oxygen (ROS) accumulation in leaves during senescence, oxidative damage occurs, especially in chloroplasts, and yellowing occurs as a result of chlorophyll breakdown [Khanna-Chopra 2012]. Chen et al. [2023] reported that exogenous ethanol application in rice seeds suppressed germination by reducing ROS signals, especially $H_2O_2$. Sako et al. [2021] reported that ethanol reduces oxidative damage caused by high light stress in *Arabidopsis thaliana* by suppressing ROS accumulation. It was determined that ROS accumulation in *Arabidopsis thaliana* and rice was inhibited by external ethanol application under salinity stress conditions, thus increasing salinity tolerance [Nguyen et al. 2017]. In the current study, it was concluded that ethanol application may be effective in delaying yellowing in spinach by suppressing the ROS accumulation that causes chloroplast degradation. Maintaining the green color of spinach means protecting its commercial value [Kaur et al. 2011]. For this reason, in this study, ethanol treatments applied to delay the yellowing of spinach succeeded in reducing green color loss. No previous study could be found regarding retarding yellowing in leafy vegetables using

ethanol treatments. Nevertheless, ethanol treatments delayed senescence in broccoli and kept the crown green [Mori et al. 2009, Xu et al. 2012]. Additionally, in mature green tomatoes, ethanol treatments slowed respiration, delayed chlorophyll breakdown due to the inhibition of ethylene synthesis, and thus delayed lycopene synthesis [Liu et al. 2019b]. Furthermore, ethanol treatments were efficient in preserving the green color of citrus that have green-colored skin [Noma et al. 2009, Opio et al. 2015]. Ethanol treatment slowed the chlorophyll breakdown in fresh-cut green beans as well [Awad et al. 2021]. It has been reported that ethanol application is effective in extending the vase life of cut cloves by suppressing the increase in ACC synthase and ACC oxidase activities [Pun et al. 2014].

Higher doses of ethanol, meanwhile, could have a harmful effect. Thus, Suzuki et al. [2004] declared that higher doses of ethanol treatments cause disruptions in broccoli. Similarly, in this study, washing spinach with water and 800 μL $L^{-1}$ ethanol caused more color loss than a 400 μL $L^{-1}$ ethanol water solution.

Ethanol treatment also reduces chlorophyll breakdown. Fukasawa et al. [2010] stated that the chlorophyll *a* and *b* contents of broccoli crowns decreased after three days of storage in the control group, while there was no significant loss in ethanol-treated broccoli. That study's result was confirmed by the current study.

Another problem in green leafy vegetables is water loss and thus withering, because the tissue properties of green leafy vegetables such as spinach directly relate to the water content and turgidity of the cell [Kanlayanarat 2009]. Additionally, leafy vegetables could be relatively perishable due to their high respiration rates and water content and wrinkling via water loss can also cause a loss of visual quality of fresh green vegetables [Brummell and Toivonen 2018]. In the present study, the ethanol applications did not prevent weight loss, while a high dose (800 μL $L^{-1}$) caused an increase in water loss. Hence, it was concluded that it is necessary to carefully use ethanol due to the risk of it causing increased water loss in leafy vegetables. The TSS content of the spinach increased significantly during storage. This increase is thought to originate from water loss. It is known that the soluble substance in cell sap rises due to intense water loss, which leads to elevated TSS. Awad et al. [2021] reported that the TSS content of fresh-cut green beans did not show crucial changes during storage. However, the spinach studied was a leafy vegetable, unlike beans, and due to its high surface/volume ratio, water loss increased, which made it seem like the amount of TSS increased.

Respiration, which is the most crucial metabolic activity of spinach during the post-harvest period, can cause changes in the carbohydrate content of the product. Moreover, a decrease in the sugar content of spinach can be connected with its utilization during respiration [Bandian et al. 2016]. However, the sugar content of spinach can increase from time to time during post-harvest storage. When a sugar analysis was conducted based on the fresh weight in products such as spinach that lose water quickly, it was discovered that the sugar concentration might change depending on cell sap. The breakdown of starch can lead to an increase in sugar content, as starch stores carbohydrates and converts them into soluble carbohydrates[Lloyd and Kötting 2016]. However, since ethanol inhibits α- and β-amylase enzyme activities, the starch does not convert to sugar, and so the sugar content can stay low [Gondi and Prasada Rao 2015]. In the current study, the high total sugar content in the control group on days 7 and 14 of storage showed that the starch converted to sugar. However, the decrease in the sugar content on storage day 7 in the spinach treated with ethanol could have resulted from the suppressing effect of ethanol on starch breakdown. It was observed that towards the end of the storage period, the effectiveness of ethanol decreased, and a high sugar content was formed, confirming the theory that ethanol inhibits starch degradation.

Methods such as UV-C [Martínez-Sánchez et al. 2019], UV-B [Kasim and Kasim 2017], and ozone application [Papachristodoulou et al. 2018] can be used to delay the yellowing of spinach and preserve its quality. However, ethanol application is a practical method since it can be used by adding it to the washing water. In spinach production, adding ethanol to the washing water during pre-marketing processing may prove useful. The utilization of minimal doses and the absence of residual ethanol risk can be regarded as crucial advantages. In addition, the fact that it does not require an infrastructure such as ultraviolet or ozone applications will increase the preferability of ethanol compared to these methods.

## CONCLUSION

According to the results of this study, the addition of ethanol to washing water before storage, is efficient in delaying both yellowing and chlorophyll loss of spinach cv. 'Matador'. The most efficient concentration of ethanol to prevent discoloration was 400 μL $L^{-1}$, while higher and lower doses of ethanol were ineffective. Moreover, the highest concentration, i.e., 800 μL $L^{-1}$, was not found to be appropriate for this purpose because it caused both increased weight loss and an ineffectiveness in delaying yellowing. In conclusion, the application of ethanol before storage can effectively reduce chlorophyll breakdown and yellowing in spinach, indicating its potential for use in other leafy vegetables.

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