

## Influence of grafting on fatty acid profile and some physicochemical properties of watermelon seed and seed oil

 B. Aydoğan-Coşkun<sup>a</sup>,  M. Ercan<sup>b</sup>,  M. Akbulut<sup>a</sup>,  H. Çoklar<sup>a,✉</sup>,  M. Seymen<sup>c</sup>,  D. Yavuz<sup>d</sup>,  
 E.S. Kurtar<sup>c</sup>,  N. Yavuz<sup>d</sup>,  S. Süheri<sup>d</sup> and  Ö. Türkmen<sup>c</sup>

<sup>a</sup>Selçuk University, Faculty of Agriculture, Department of Food Engineering, Konya, Turkey

<sup>b</sup>Bingöl University, Faculty of Engineering and Architecture, Department of Food Engineering, Bingöl, Turkey

<sup>c</sup>Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Turkey

<sup>d</sup>Selçuk University, Faculty of Agriculture, Department of Irrigation, Konya, Turkey

✉Corresponding author: [hacercoklar@selcuk.edu.tr](mailto:hacercoklar@selcuk.edu.tr)

Submitted: 19 July 2021; Accepted: 14 September 2021

**SUMMARY:** This study aimed to investigate the effects of grafting on the fatty acid profile and some physicochemical properties of watermelon seed and seed oil. The ‘Crimson Tide’ cultivar was used as the scion while two wild watermelon (*Citrullus lanatus* var. *citroides* (A1 and A2)), one *Lagenaria siceraria* (A3) and one *Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne (A4) were used as rootstocks. The use of rootstock significantly influenced the fatty acid profile and the physical parameters of seeds and seed oils. The highest linoleic acid ratio was found in the seed oil from A1 and A2, the oil from A3 had the highest oleic acid ratio. The results showed that the content and acid value in seed oils were improved, and that total phenolic compounds and antioxidant activity of both seed and oil were decreased by grafting. Wild rootstocks can be used in watermelon cultivation to obtain a watermelon seed which is rich in linoleic acid.

**KEYWORDS:** Citron watermelon; Grafting; PCA; Rootstock; Watermelon seed; Watermelon seed oil

**RESUMEN:** *Influencia de un injerto en el perfil de ácidos grasos y algunas propiedades fisicoquímicas de la semilla y el aceite de semillas de sandía.* El objetivo de este estudio fue investigar los efectos del injerto en el perfil de ácidos grasos y algunas propiedades fisicoquímicas de la semilla y el aceite de semillas de sandía. El cultivar ‘Crimson Tide’ se utilizó como vástago, mientras que dos sandías silvestres (*Citrullus lanatus* var. *Citroides* (A1 y A2)), una *Lagenaria siceraria* (A3) y una *Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne (A4) se utilizaron como portainjertos. El uso de portainjertos influyó significativamente en el perfil de ácidos grasos y los parámetros físicos de semillas y aceites de semillas. La proporción de ácido linoleico más alta se encontró en el aceite de semillas de A1 y A2, el aceite de A3 tuvo la proporción de ácido oleico más alta. Los resultados mostraron que el contenido de aceite y el índice de acidez mejoró y los compuestos fenólicos totales y la actividad antioxidante tanto de la semilla como del aceite se redujeron mediante el injerto. Para obtener un aceite de semillas de sandía rico en ácido linoleico, se pueden utilizar portainjertos silvestres en el cultivo de sandía.

**PALABRAS CLAVE:** Aceite de semilla de sandía; Injerto; PCA; Portainjerto; Sandía cidra; Semilla de sandía

**Citation/Cómo citar este artículo:** Aydoğan-Coşkun B, Ercan M, Akbulut M, Çoklar H, Seymen M, Yavuz D, Kurtar ES, Yavuz N, Süheri S, Türkmen Ö. 2022. Influence of grafting on fatty acid profile and some physicochemical properties of watermelon seed and seed oil. *Grasas y Aceites* 73 (3), e475. <https://doi.org/10.3989/gya.0784211>

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## 1. INTRODUCTION

The watermelon (*Citrullus lanatus*), which belongs to the family Cucurbitaceae, is one of the most nutritionally and economically important horticultural crops worldwide (Acar *et al.*, 2012; Kombo and Sari, 2019). According to data from the Food and Agriculture Organization (FAO), a total of 100,414,933 tons per year of watermelon was produced in the world (FAOSTAT, 2020). China (60.4%) is the leading producer of watermelon, followed by Turkey (3.9%) and India (2.5%). Most of the fruit's seeds are usually discarded, and some are considered by-products. The seeds, which constitute 1-4% of the total watermelon weight, are highly nutritious and are rich in protein, vitamin B, minerals and oil (Braide *et al.*, 2012; Duduyemi *et al.*, 2013; Kiin-Kabari and Akusu, 2014; Seidu and Otutu, 2016; Tabiri *et al.*, 2016). Watermelon seed oil, whose content value is generally between 19-35% is used as edible oil in the food industry and as an ingredient in the cosmetic industry (Jensen *et al.*, 2011; Tabiri *et al.*, 2016; Rezig *et al.*, 2019). Unsaturated fatty acids (UFAs) are dominant in the fatty acid profile of watermelon seed oil. The saturated (mainly palmitic (16:0) and stearic (18:0) acids), monounsaturated (mainly oleic acid (18:1( $\omega$ -9)) and polyunsaturated (mainly linoleic acid (18:2( $\omega$ -6)) fatty acid contents in the seed oil are generally 17.90, 16.31 and 65.79%, respectively (de Conto *et al.*, 2011; Eke *et al.*, 2021).

The quality of an oil obtained from a vegetable or seed is highly related to its unsaturated fatty acid profile (Nayeri and Yarizade, 2014). The proportions of oleic and linoleic acids in edible oils are generally indicative of their oxidative stability and nutritional properties (Nayeri and Yarizade, 2014). Polyunsaturated fatty acids are the most beneficial for the preservation of human health and for controlling diseases such as cancer, inflammation, rheumatoid arthritis, cardiovascular disorders, and coronary heart diseases. In fact, the higher the degree of unsaturation in  $\omega$ -3, 6, 9 fatty acids, the more positive are the effects on human health. Another important aspect of linoleic acid is that it is essential and must be consumed because it cannot be synthesized in the human body (Asif, 2015). Grafting plants on coherent rootstocks ensures some advantages like augmenting plant vig-

or, prolonging harvesting period and post-harvest life, providing water efficiency and tolerance to drought and salt (Yetisir *et al.*, 2003; Davis *et al.*, 2008; Lee *et al.*, 2010; Yetisir and Uygur, 2010; Zhao *et al.*, 2011; Kumar *et al.*, 2017; Solmaz *et al.*, 2018). Several studies stated that grafting alters the content and quality of fruit. That being said, for successful grafting, choosing suitable rootstock is important (Davis *et al.*, 2008a; Davis *et al.*, 2008b; Turhan *et al.*, 2012). Grafting is commonly used in watermelon cultivation. *Cucurbita maxima*  $\times$  *Cucurbita moschata* hybrids and *Lagenaria siceraria* are the most common commercial rootstocks. On the other hand, wild watermelons (*Citrullus lanatus* var. *citroides*) are important gene sources and are used in rootstock and breeding studies.

Although many research studies regarding the effects of grafting on watermelon seed content are available, there has been no report on the analysis in terms of fatty acid profile and some physicochemical properties (except thousand grain weight) of seeds and seed oils from grafted watermelons in the literature. The aim of this study was to determine the effect of grafting on the seed and the seed oil of the Crimson Tide variety, which was grafted on *Cucurbita maxima*  $\times$  *Cucurbita moschata*, *Lagenaria siceraria* and two wild watermelon rootstocks. In this context, the effects of grafting, namely total phenolic compounds, antioxidant activity and reflectance color values ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h$ ) of the watermelon seeds and seed oils were evaluated. Also, thousand grain weight and width-length-thickness dimensions of the seeds, and seed oil content, kernel oil content, fatty acid profile, and acid value of the seed oils were investigated.

## 2. MATERIALS AND METHODS

### 2.1. Plant material and planting

The research was carried out in Selçuk University, Faculty of Agriculture, Sarıcalar Research and Application Farm, in 2017. The trial zone is located between 38° 05' northern latitudes and 32° 36' east longitudes and its average height from the sea is 1006 m. In the study, two wild rootstocks *Citrullus lanatus* var. *citroides* (A1 and A2), one open-pollinated *Lagenaria siceraria* (A3) and one *Cucurbita maxima* Duchesne  $\times$  *Cucurbita moschata* Duchesne hybrid ('TZ-148') (A4) were used as rootstocks.

'Crimson Tide' (CT), a commercial watermelon variety widely grown in Turkey, was grafted on these four rootstocks and production materials were obtained. Ungrafted CT (K) was used as control. A total of 5 trial subjects were used in three replications according to the randomized block design. All cultural practices up to the harvest were applied to all applications equally. Watermelon fruits were harvested on time and after harvest, watermelon seeds from each application were hand collected separately, washed to remove the fruit pulp residue and dried in an environment free from sunlight at room temperature for a week. The seeds were kept in freezer bags at 4 °C until oil extraction and analyses.

## 2.2. Determination of thousand grain weight and seed size

Seeds (50 samples) were counted and weighed, and the weight was multiplied by 20 to obtain a thousand grain weight (Pradhan, 2010). The width, length and thickness of the seed grains were determined in mm using a caliper.

## 2.3. Determination of oil content in seeds and kernels

According to AOCS (1980) with some modifications, approximately 2 g of the milled seeds and kernels (shelled seeds) were extracted using 250 ml of petroleum ether in the Soxhlet device for 4 hours. The solvent was removed from the miscella by a rotary vacuum evaporator (RE100-Pro, Scilogex, CT, USA) set at 40 °C, and the oil contents of the samples were calculated.

## 2.4. Determination of acid numbers in seed oils

The acid number analysis was determined by making some modifications according to TSI (2003). Firstly, 25 ml of ethyl alcohol-diethyl ether mixture (1:1) were added to 2 g oil sample. The mixture was titrated with a 0.1 N ethanolic NaOH solution with 1 ml phenolphthalein indicator. Results were given as mg NaOH/g oil.

## 2.5. Determination of fatty acid profile of seed oils

The fatty acid methyl esters of seed oils were prepared according to the method described by Williams (1984) with some modifications. The sample was shaken vigorously in isooctane (0.4 g in 4 ml)

and combined with 0.2 ml of 2 N methanolic potassium hydroxide. After the duration in the dark for 6 min, a drop of methyl orange and 0.45 ml of 1 N HCl were added, mixed and centrifuged (2000 rpm/5 min). The supernatants were then analyzed by a GC (Agilent, Santa Clara, CA) equipped with an injector, a capillary column (Innowax, 100 m length, 0.25 mm i.d., 0.20 µm of thickness), and a flame ionization detector (FID). Hydrogen was used as carrier gas. The temperature of the detector and injector was set as 250 °C. The initial oven temperature was 180 °C and increased to 220 °C at 30 °C/min.

## 2.6. Determination of reflectance color values of seeds and seed oils

Color measurements of the seeds and their oils were determined as  $L^*$  (lightness-darkness),  $a^*$  (redness-greenness),  $b^*$  (yellowness-blueness),  $C^*$  (chroma) and  $h$  (hue) values in the CIE color system profile using Minolta colorimeter (CM-5, Minolta, Japan).

## 2.7. Extraction of polyphenols and antioxidant effective compounds from seeds and seed oils

To extract the polyphenols and antioxidant effective compounds from seeds, 0.1 g of defatted ground seed obtained at the end of the oil extraction was weighed and 40 ml of 80% methanol solution were added. Homogenization was obtained by an ultra-turrax operation for 2 minutes at 200 rpm. Similarly, 1 g of oil sample was mixed with 2 ml methanol for 10 minutes in a shaker. The supernatants of oil-methanol and seed-methanol obtained from the centrifugation at 4100 rpm for 10 minutes was used for total phenolic compounds and antioxidant activity analyses.

## 2.8. Determination of total phenolic compounds

The total phenolic compounds (TPC) of oils and seeds were obtained using the Folin-Ciocalteu method (Singleton and Rossi, 1965), with slight modifications. The Folin-Ciocalteu reagent of 1.25 ml (0.2 N) and 1 ml of sodium carbonate (75 g/l) solution were added to 250 µl of extract. Before determining the absorbance values of samples by spectrophotometer at 765 nm, they were kept in the dark for 2 hours. Results were expressed as mg gallic acid equivalent (GAE)/kg.

TABLE 1. The thousand grain weight, dimensions, total phenolic compounds and antioxidant activity values of the watermelon seeds

Rootstocks	Thousand grain weight (g)	Width (mm)	Length (mm)	Thickness (mm)	Total phenolic compounds (mg GAE/kg)	Antioxidant activity (mmol TE/kg)
K	44.00±0.20 <sup>b</sup>	5.36±0.10 <sup>b</sup>	8.87±0.17 <sup>a</sup>	1.95±0.03 <sup>a</sup>	2340.87±17.39 <sup>a</sup>	6.80±0.53 <sup>a</sup>
A1	40.70±0.30 <sup>d</sup>	5.07±0.10 <sup>c</sup>	8.55±0.19 <sup>b</sup>	1.70±0.11 <sup>b</sup>	1366.96±121.74 <sup>d</sup>	5.22±0.53 <sup>b</sup>
A2	41.50±0.10 <sup>c</sup>	5.22±0.13 <sup>bc</sup>	8.63±0.08 <sup>b</sup>	1.72±0.05 <sup>b</sup>	1575.65±86.96 <sup>c</sup>	6.54±0.26 <sup>a</sup>
A3	48.70±0.10 <sup>a</sup>	5.77±0.12 <sup>a</sup>	9.05±0.14 <sup>a</sup>	1.91±0.04 <sup>a</sup>	1140.87±110.00 <sup>e</sup>	3.57±0.99 <sup>d</sup>
A4	42.20±0.80 <sup>c</sup>	5.04±0.07 <sup>c</sup>	8.38±0.11 <sup>b</sup>	1.66±0.08 <sup>b</sup>	1801.74±139.13 <sup>b</sup>	4.16±0.53 <sup>c</sup>

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

TABLE 2. Color values of watermelon seeds

Rootstocks	L*	a*	b*	C*	h
K	37.73±1.07 <sup>a</sup>	5.25±0.28 <sup>a</sup>	12.92±1.11 <sup>a</sup>	67.77±1.00	13.92±1.15 <sup>a</sup>
A1	31.42±2.46 <sup>bc</sup>	3.39±0.56 <sup>b</sup>	7.65±1.74 <sup>b</sup>	65.90±1.93	8.37±1.81 <sup>b</sup>
A2	31.56±1.32 <sup>bc</sup>	3.17±0.51 <sup>b</sup>	7.38±1.33 <sup>b</sup>	66.72±0.95	8.04±1.42 <sup>b</sup>
A3	29.77±1.52 <sup>c</sup>	3.69±0.13 <sup>b</sup>	7.45±0.87 <sup>b</sup>	63.49±2.15	8.31±0.82 <sup>b</sup>
A4	33.65±1.89 <sup>b</sup>	3.93±0.55 <sup>b</sup>	8.46±1.41 <sup>b</sup>	64.99±0.68	9.33±1.51 <sup>b</sup>

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

## 2.9. Determination of antioxidant activity

The antioxidant activity of watermelon seeds and their oils was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, according to an adapted colorimetric procedure (Akbulut *et al.*, 2009). After adding 1.95 ml of DPPH reagent to 50 µl extract, the mixture was kept in the dark for 30 minutes. The absorbance values of the samples were measured by spectrophotometer at 515 nm and antioxidant activity values were calculated in mmol Trolox Equivalent (TE)/kg.

## 2.10. Statistical analysis

Significant differences among samples were determined through analysis of variance (ANOVA) and Duncan's multiple range test using SPSS package program version 22.0. Differences were considered significant at p < 0.01. The JMP 13.0 software was used to analyze the important parameters by Principal Component Analysis (PCA). The relation between rootstocks was compared by drawing a loading plot graph using the same software and the relationship between each parameter and another was presented on the score plot graph.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of grafting on watermelon seeds

Some physicochemical properties of the seeds obtained from watermelon fruits are given in Tables 1 and 2. According to Table 1, the rootstock had statistically significant effects on thousand grain weight, width-length-thickness dimensions, total phenolic compounds (TPC) and antioxidant activity (AA) values for the seeds. Thousand grain weight results of seeds ranged from 40.70 g to 48.70 g, and watermelons grafted on the bottle gourd rootstock (A3) with the highest thousand grain weight were statistically separated from other samples according to Duncan test results. While the thousand grain weight of the seeds of watermelons grafted on Citroides 1 (A1), Citroides 2 (A2) and TZ148 (A4) rootstocks was significantly decreased, that of watermelon seeds grafted on the bottle gourd (A3) was significantly increased (p < 0.01). Similar to the results of this study, the grafting of Crimson Sweet (CS) watermelon (*Citrullus lanatus*) steel on three different rootstocks (*Cucurbita maxima* x *Cucurbita moschata* 'NUN-9075', *Lagenaria siceraria* 'Argentario', and citron watermelon *Citrullus amarus*

TABLE 3. Oil content in seeds and some physicochemical, and phytochemical properties of watermelon seed oils

Rootstocks	Oil content (%)	Kernel oil content (%)	Acid value (mg NaOH/g)	Total phenolic compound (mg GAE/kg)	Antioxidant activity (mmol TE/kg)
K	24.05±0.19 <sup>d</sup>	45.61±0.11	5.35±0.20 <sup>a</sup>	51.86±0.26	0.52±0.03 <sup>a</sup>
A1	25.90±0.77 <sup>ab</sup>	46.44±0.34	4.33±0.19 <sup>d</sup>	50.69±0.65	0.45±0.01 <sup>bc</sup>
A2	26.34±0.38 <sup>a</sup>	44.19±0.84	4.86±0.06 <sup>b</sup>	53.56±1.96	0.44±0.01 <sup>c</sup>
A3	24.75±0.36 <sup>cd</sup>	44.44±1.52	4.76±0.13 <sup>bc</sup>	51.50±0.68	0.46±0.01 <sup>bc</sup>
A4	25.46±0.35 <sup>bc</sup>	45.19±0.99	4.50±0.15 <sup>cd</sup>	49.25±2.87	0.49±0.01 <sup>ab</sup>

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test.

TABLE 4. The fatty acid profile (%) of watermelon seed oils

Rootstocks	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2
K	11.90±0.00 <sup>b</sup>	7.97±0.00 <sup>a</sup>	16.74±0.41 <sup>c</sup>	63.40±0.40 <sup>b</sup>
A1	11.63±0.11 <sup>c</sup>	7.59±0.11 <sup>b</sup>	15.98±0.11 <sup>d</sup>	64.81±0.33 <sup>a</sup>
A2	12.27±0.00 <sup>a</sup>	7.27±0.15 <sup>c</sup>	15.78±0.02 <sup>d</sup>	64.68±0.13 <sup>a</sup>
A3	12.25±0.08 <sup>a</sup>	7.95±0.14 <sup>a</sup>	17.86±0.16 <sup>a</sup>	61.88±0.00 <sup>c</sup>
A4	11.96±0.11 <sup>b</sup>	7.40±0.01 <sup>bc</sup>	17.33±0.07 <sup>b</sup>	63.32±0.04 <sup>b</sup>

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test. Standard deviation values below 0.01 were equal to ± 0.00

Schard 'PI296341') had a significant effect on the thousand grain weight of the seeds obtained (Kombo and Sari, 2019).

As seen in Table 1, the width, length and thickness of the watermelon seeds varied from 5.0-5.8 mm, 8.4-9.0 mm and 1.7-1.9 mm, respectively. A decrease in the total amount of phenolic compounds of all watermelon seeds due to grafting was observed. It was determined that the seeds with the highest TPC belonged to the control sample (2340.9 mg GAE/kg) and the lowest to the A3 sample (1140.9 mg GAE/kg). In an analysis of the seeds of *Citrullus lanatus* var. *citroides*, Acar *et al.* (2012) found that the TPC of the seeds were 130 mg GAE/kg, and the reason for the decrease in the TPC of A1 and A2 samples may be due to the low content in the citroides species. Many recent studies (Seidu and Otutu, 2016; Tabiri *et al.*, 2016) have shown that watermelon seeds have much more TPC than those of this study. These differences in TPC may depend on factors such as variety and environmental conditions. The antioxidant activity values of watermelon seeds varied between 3.57 and 6.80 mmol TE/kg. As in the amount of phenolic compounds, a statistically significant decrease (except A2 sample) was observed in the antioxidant activity values of the seeds obtained as a result of

grafting. Tabiri *et al.* (2016) reported the antioxidant activity values of the seeds of "Charleston gray", "Black diamond" and "Crimson sweet" watermelon varieties as 82.59, 96.63 and 130.29 mmol TE/kg, respectively.

The decrease in color values of the watermelon seeds was found to be significant for  $L^*$ ,  $a^*$ ,  $b^*$  and  $h$ , but not for  $C^*$  (Table 2). The  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and  $h$  values varied between 29.77-37.73, 3.17-5.25, 7.38-12.92, 63.49-67.77 and 8.04-13.92, respectively. If we expanded the results for watermelon seeds, no significant difference was observed only in the  $C^*$  values after grafting.

### 3.2. Effect of grafting on watermelon seed oil

The seed oil content, kernel oil content, acid value, TPC and AA values of the samples are presented in Table 3 and the fatty acid profile in Table 4. It has been determined that the differences that occurred in these values except for the kernel oil content and TPC results are statistically significant ( $p < 0.01$ ). The increase observed in the oil efficiency of all the seeds by grafting was found statistically significant. Sample A2 (26.34%) had the highest seed oil content, followed by A1 (25.90%), A4 (25.46%),

TABLE 5. Color values of watermelon seed oils

Rootstocks	L*	a*	b*	C*	h
K	87.09±0.01 <sup>c</sup>	-0.47±0.00 <sup>e</sup>	80.37±0.01 <sup>a</sup>	80.37±0.01 <sup>a</sup>	90.34±0.00 <sup>c</sup>
A1	88.08±0.01 <sup>d</sup>	-0.60±0.02 <sup>d</sup>	74.63±0.03 <sup>c</sup>	74.64±0.04 <sup>c</sup>	90.46±0.01 <sup>d</sup>
A2	88.30±0.03 <sup>c</sup>	-0.75±0.01 <sup>c</sup>	77.43±0.01 <sup>b</sup>	77.43±0.01 <sup>b</sup>	90.56±0.01 <sup>c</sup>
A3	88.60±0.03 <sup>b</sup>	-1.08±0.02 <sup>b</sup>	74.60±0.00 <sup>c</sup>	74.61±0.00 <sup>c</sup>	90.83±0.01 <sup>b</sup>
A4	89.08±0.04 <sup>a</sup>	-1.21±0.01 <sup>a</sup>	62.11±0.03 <sup>d</sup>	62.12±0.02 <sup>d</sup>	91.12±0.01 <sup>a</sup>

The results are expressed as means ± standard deviation of 3 replicates (n = 3). Different letters in the same column indicate significant differences (p < 0.01) among rootstocks according to the one-way ANOVA/ Duncan's multiple range test. Standard deviation values below 0.01 were equal to ± 0.00

TABLE 6. PCA results regarding some seed characteristics of scion in different rootstocks

Items	PC1	PC2
Eigenvalue	5.91	4.15
Percentage of variance	53.7	37.7
Cumulative variance	53.7	91.4
Eigenvectors		
Thousand grain weight	-0.01574	<b>0.48529</b>
Width	0.00410	<b>0.47570</b>
Length	0.13154	<b>0.43907</b>
Thickness	0.19524	<b>0.43071</b>
L*	<b>0.39446</b>	-0.09372
a*	<b>0.38036</b>	0.12672
b*	<b>0.40644</b>	0.04465
C*	<b>0.30853</b>	-0.25675
h	<b>0.40444</b>	0.05557
Total phenolic compounds	<b>0.38396</b>	-0.13156
Antioxidant activity	0.26956	-0.21624

PC1 and PC2 indicate first and second components, respectively.

A3 (24.75%) and lastly K (24.05%). However, there was no similarity between seed oil content and kernel oil content in the samples. Kernel oil content values were listed in descending order as follows: A1, K, A4, A3, A2. The oil contents in the seeds and kernels of *Citrullus lanatus* var. *citroides* were reported by Acar *et al.* (2012) as 26.83 and 52.34%, respectively. In addition, four *Lagenaria siceraria* cultivars were studied by Essien *et al.* (2013) and the content in seed oils, which is close to our results, was obtained in the range of 23.0-29.5%. Tabiri *et al.* (2016) reported that the seed oil contents in "Crimson sweet", "Charleston gray" and "Black diamond" varieties were 26.50, 26.83 and 27.85%, respectively. In one of the studies, the seed oil content values

of the "Crimson" cultivar was 19.23% (de Conto *et al.*, 2011), while in another, in the "Mateera" and "Sugar baby" varieties were 25.53% (46.85% kernel oil content) and 21.93% (38.88% kernel oil content), respectively (Wani *et al.*, 2013). Considering all of this evidence, it seems that oil content values can vary based on the watermelon variety.

The acid values for the samples (4.33 to 5.35 mg NaOH/g) were relatively high compared to that reported for watermelon (*Citrullus lanatus* L.) seeds (2.37 mg NaOH/g) (Duduyemi *et al.*, 2013). With the effect of grafting, there was a significant decrease in acid value as given in Table 3 (p < 0.01). Among the grafted rootstocks, A2 (4.86 mg NaOH/g) had the highest acid value, while A1 (4.33 mg NaOH/g) had the lowest. Essien *et al.* (2013) determined the acid values for the seed oils from four gourd cultivars as between 2.1-2.4 mg KOH/g. For this value, the decrease in A3 compared to the control sample may be due to the low acidity of the oil from the gourd seeds. Wani *et al.* (2013) obtained oil from "Mateera" and "Sugar baby" cultivars and reported that the acid values in mg KOH/g oil were 4.27 and 6.46, respectively. In another investigation, in which cold press was applied, the acid value of the "Crimson" variety seed oil was found to be 9.5 mg KOH/g oil (Rezig *et al.*, 2019). The TPC of the seed oils from grafted (49.25-53.56 mg GAE/kg) and ungrafted (51.86 mg GAE/kg) watermelons was lower than that of Iranian watermelon seed oils (111 mg GAE/kg), as reported by Hashemi *et al.* (2017). In addition, the TPC of the seed oils from four gourd cultivars was found to be between 80 and 105 mg GAE/kg as reported by Essien *et al.* (2015). The decrease observed in the antioxidant activity of oils by grafting was found statistically significant (p < 0.01). The control sample (K) (0.52 mmol TE/kg) had the highest antioxidant

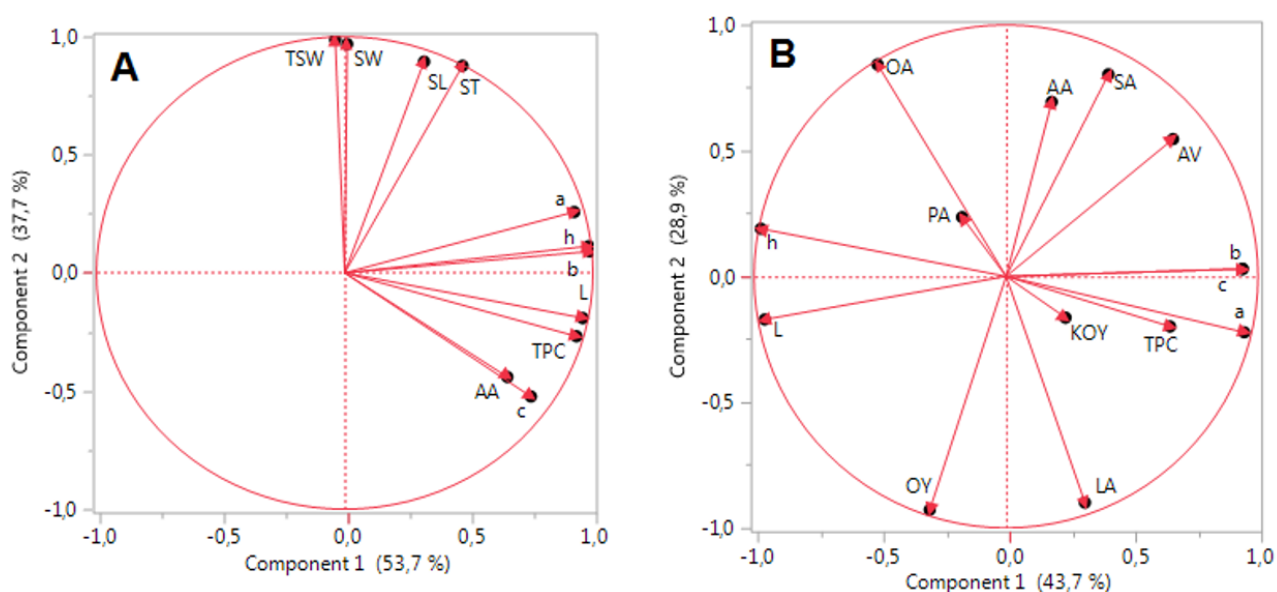


FIGURE 1. Loading plot based on PC1 and PC2 obtained by principal component analysis using some seed (A) and oil (B) characteristics of scion on different rootstocks

\*PC1: First component; PC2: Second component; OY (Oil yield); KOY (Kernel oil yield); TPC (Total phenolic compounds); AA (Antioxidant activity);  $L^*$  (Lightness);  $a^*$  (redness-greenness);  $b^*$  (yellowness-blueness);  $C^*$  (Chroma); h (Hue); AV (Acid Value); LA (Linoleic Acid); PA (Palmitic Acid); SA (Stearic Acid); OA (Oleic Acid); TSW (thousand grain weight); SW (width); SL (length); ST (thickness)

activity, followed by the A4 (0.49 mmol TE/kg), A3 (0.46 mmol TE/kg), A1 (0.45 mmol TE/kg), A2 (0.44 mmol TE/kg) samples.

The polyunsaturated, monounsaturated and saturated fatty acid profiles of the watermelon seed oils statistically changed with the application of grafting ( $p < 0.01$ ) (Table 4). The fatty acid distribution in the ungrafted control sample (Crimson Tide) was as follows: 63.40% linoleic acid, 16.74% oleic acid, 11.90% palmitic acid and 7.97% stearic acid. When compared to the control sample, an increase in the amount of unsaturated fatty acids (UFAs) in the A1, A2 and A4 samples and of saturated fatty acids (SFAs) in the A3 was observed. The highest linoleic acid content was determined in the seed oils from the citroides rootstock samples as 64.81% (A1) and 64.68% (A2), the lowest oleic acid content was also determined in the same samples as 15.98% and 15.78%, respectively. According to the control sample, the grafting application in citroides samples caused a statistically significant increase in linoleic acid content, with a significant decrease in the oleic acid content. The seed oil from the bottle gourd rootstock sample (A3) had the highest oleic acid content (17.86%), but the lowest linoleic acid (61.88%). No work regarding the effect of grafting on the fatty acid profile of the seed oils from watermelons or the other plants in the Cucurbitace-

ae family could be found. This situation is also valid for all analyses performed on the seeds and their oils except thousand grain weight. The obtained results for the fatty acid profiles show similarity with the studies performed by Wani *et al.* (2013), Hashemi *et al.* (2017), Rezig *et al.* (2019), and Eke *et al.* (2021). With the effect of grafting, a significant difference was observed in the color values of watermelon seed oils (Table 5). The increase in  $L^*$  and h values and the decrease in  $a^*$ ,  $b^*$  and  $C^*$  values were statistically significant ( $p < 0.01$ ). The values for  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  and h in the oils were between 87.09-89.08, (-)0.47-(-)1.21, 62.11-80.37, 62.12-80.37 and 90.34-91.12, respectively.

### 3.3. Principal component analysis (PCA)

#### 3.3.1. PCA of seeds

PCA is a practical and commonly used method to estimate the agricultural practices on product content and quality (Seymen *et al.*, 2019). The seeds of the watermelon grafted with different rootstocks, thousand grain weight, width, length, thickness, color values ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ , h), total phenolic compounds and antioxidant activity results obtained were subjected to PCA (Table 6). As a result of PCA, the study explained a high rate of 91.4%

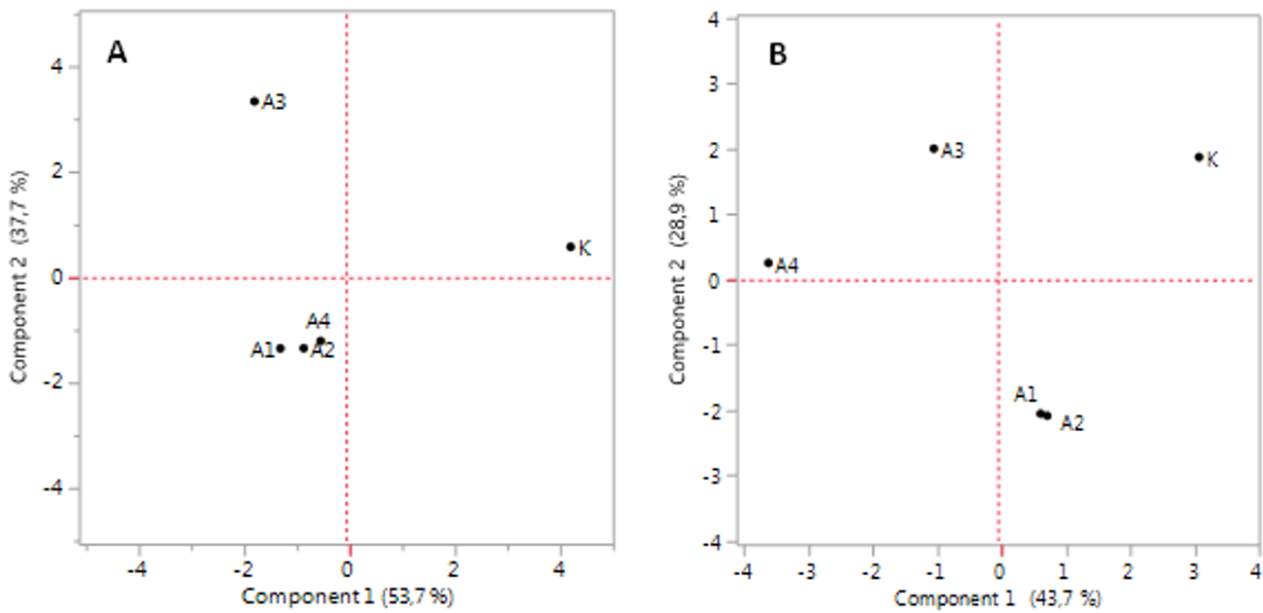


FIGURE 2. Score plot based on PC1 and PC2 obtained from principal component analysis using some seed (A) and oil (B) characteristics of scion on different rootstocks

\*PC1: First component; PC2: Second component; K (Control); A1 and A2 (*Citrullus lanatus* var. *citroides*); A3 (*Lagenaria siceraria*); A4 (*Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne hybrid ('TZ-148'))

in two components. Seymen *et al.* (2019) reported that the first two components should be explained by more than 25% of the study to use PCA. According to the results obtained, 91.4% of two components studied were detected and it was revealed that this analysis will give important results about the usability and the parameters examined. The first component (PC1) explained 53.7% of the study and  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h$  and total phenolic compound parameters were the highest declared parameters positively. The second component (PC2) explained 37.7% of the study and thousand grain weight, width, length, and thickness parameters were the highest explained parameters.

A loading chart was created to examine the relationships among thousand grain weight, width, length, thickness color ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h$ ), total phenolic compounds and antioxidant activity (Figure 1-A). If the angle between the vectors in the figure is  $< 90^\circ$ , there is a positive relationship; if it is  $> 90^\circ$ , there is a negative relationship; and if it is equal to  $90^\circ$ , there is no relationship. As seen in the figure, the highest positive relationships were seen among antioxidant activity- $C^*$ ,  $h$ - $b^*$  and thousand grain weight-width. Similarly, Seymen *et al.* (2019) reported positive relation-

ships between thousand grain weight-seed width and thousand grain weight-length in pumpkin seeds. On the other hand, the dimensions of seeds and thousand grain weight parameters showed no relationship with seed color or other chemical contents. In the score plot (Figure 2-A), control (K) was in the positive region of both components, which showed significant results for the parameters in both components. As a result, the control had significant effects on some physico-chemical properties of watermelon seeds. On the other hand, wild rootstocks (A1 and A2) and A4 showed similarity in terms of seed characteristics and were located in the same region on the plot.

### 3.3.2. PCA of seed oil

The seed oil from the watermelon grafted with different rootstocks, oil content, kernel oil content, total phenolic compounds, antioxidant activity, color values ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h$ ), acid value, stearic acid, palmitic acid, oleic acid and linoleic acid results obtained were subjected to PCA (Table 7). As a result of PCA, the study explained a high rate of 94.07% in three components.

According to the results obtained, 94.1% of three components studies were detected and it was



revealed that this analysis will give important results about the usability and the parameters examined. The first component (PC1) and the second component (PC2) explained 43.7 and 28.9% of the study, respectively.  $a^*$ ,  $b^*$ , and  $C^*$  parameters in PC1 were the highest declared parameters positively while  $L^*$  and  $h$  parameters were the highest declared negatively. In PC2 antioxidant activity, oleic acid and stearic acid were the highest parameters declared positively; oil content, and linoleic acid were the highest parameters declared negatively. The third component (PC3) explained 21.5% of the study and total phenolic compounds and palmitic acid parameters were the highest positive parameters and kernel oil content was the highest negative parameter.

A loading chart was created to examine the relationships among oil content, kernel oil content, total phenolic compounds, antioxidant activity, color ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h$ ), acid value, stearic acid, palmitic acid, oleic acid and linoleic acid (Figure 1-B). The highest positive relationships were found between  $a^*$ - total phenolic compounds,  $b^*$ - $C^*$ . The highest negative relationships were found between linoleic acid-oleic acid, oil content-antioxidant activity. Similar to the result of this research, Gangadhara and Nadaf (2018) reported strong negative relationships between oleic acid and linoleic acid in two backcross generations of groundnut. Delta-12 fatty acid desaturase 2 (FAD2) enzyme, which is specific to plant seeds, converts oleic acid to linoleic acid by desaturation and leads to high linoleic acid contents in some seed oils (Nayeri and Yarizade, 2014). The negative relationship between oleic and linoleic acids in this study may be explained by conversions among fatty acids. Higher linoleic acid ratios in the oils of A1 and A2 samples can arise from the activities of such enzymes. In the score plot, control (K) was in the positive region of both components (Figure 2-B). As a result, the control had significant effects on the some physicochemical properties of watermelon seed oil. However, A1 and A2 wild rootstocks differed from other rootstocks and the control sample with its high linoleic acid content. When taking into consideration the importance of linoleic acid for human health and linoleic acid ratios of A1 and A2 samples, wild watermelon rootstocks can be an alternative

TABLE 7. PCA results regarding some oil characteristics of scion in different rootstocks

Items	PC1	PC2	PC3
Eigenvalue	6.11	4.04	3.02
Percentage of variance	43.7	28.9	21.5
Cumulative variance	43.7	72.5	94.1
Eigenvectors			
Oil content	-0.12247	<b>-0.46143</b>	0.12545
Kernel oil content	0.09545	-0.08155	<b>-0.54450</b>
Total phenolic compounds	0.26307	-0.09902	<b>0.42191</b>
Antioxidant activity	0.07336	<b>0.34514</b>	-0.26465
$L^*$	<b>-0.38725</b>	-0.08516	0.11911
$a^*$	0.38234	-0.11057	-0.13621
$b^*$	<b>0.37950</b>	0.01483	0.16105
$C^*$	<b>0.37947</b>	0.01485	0.16096
$h$	<b>-0.39349</b>	0.09420	0.05256
Acid Value	0.26798	0.27154	0.16867
Palmitic acid	-0.07043	0.11719	<b>0.54856</b>
Stearic acid	0.16425	<b>0.39950</b>	-0.09502
Oleic acid	-0.20623	<b>0.41906</b>	0.01803
Linoleic acid	0.12656	<b>-0.44726</b>	-0.11687

PC1, PC2, and PC3 indicate first, second, and third components, respectively.

to commercial rootstocks in watermelon grafting in terms of seed oil production.

#### 4. CONCLUSIONS

The present study demonstrated that the application of grafting to watermelon significantly influenced the fatty acid profile and physicochemical properties of seeds and seed oils apart from  $C^*$  values for the seeds, kernel oil content and total phenolic compounds of seed oils. Although the use of rootstocks decreased the total phenolic compounds and the antioxidant activity of the seeds, it increased the oil efficiency of the seeds and improved the use of them for seed oil. In addition, the use of citroides rootstocks can be recommended in terms of high content in linoleic acid. This study is the first to investigate the detailed characteristics of grafted watermelon seeds. Therefore, more studies are needed on this subject.

## ACKNOWLEDGMENTS

This research was funded by the Scientific Research Projects Coordination Office of Selçuk University, Turkey (Project Number 18401007).

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