

ANTIOXIDANT ACTIVITY, SOME NUTRITIONAL AND COLOUR PROPERTIES OF VACUUM DRIED STRAWBERRY TREE (*ARBUTUS UNEDO* L.) FRUIT

H. Hülya Orak¹, Türkan Aktas¹, Hülya Yagar², S. Selen Isbilir², Neslihan Ekinci³, Fusun H. Sahin¹

¹Namık Kemal University in Tekirdağ, Turkey

²Trakya University in Edirne, Turkey

³Çanakkale (18th March) University, Turkey

Background. The strawberry tree (*Arbutus unedo* L.) fruit contains a higher amount of nutrients and bioactive compounds than many other cultivated species, however, the edible use of this fruit is currently not widespread. In this study, the influences of vacuum drying have been investigated in terms of changing of some nutritional characteristics, antioxidant properties, and colour.

Material and methods. Fruits were collected from Çanakkale province in Turkey and next vacuum dried. Ethyl oleate and water blanching pre-treatments were applied to fruits before drying. Ascorbic acid, reducing sugar, minerals, colour, total phenolic content, DPPH radical scavenging activity, β -carotene bleaching activity and HMF formation were determined.

Results. The EO pretreatment shortened the drying time more than WB and gives a higher β -carotene bleaching activity, lower HMF and higher yellowness and brightness of external colour characteristics.

Conclusions. In this research, the effects of vacuum drying process on the colour, antioxidant activity and nutritional characteristics of fruit have been determined and it has been concluded that the strawberry tree fruit is assessable in food industry by drying due to rich nutritional components, antioxidant activity and attractive colour of the fruit.

Key words: *Arbutus unedo* L., DPPH activity, ascorbic acid, minerals, colour, pretreatments, vacuum drying

INTRODUCTION

Arbutus unedo L., the strawberry tree (Ericaceae family) fruit, is an evergreen shrub or small tree and it is widely distributed in the Mediterranean region and North Africa. This fruit is suitable for the production of alcoholic beverages, jams, jellies and marmalades [Pallauf et al. 2008] and in some countries, such as Spain and Morocco, *Arbutus unedo* is frequently used in the traditional medicine [Tahraoui et al. 2007].

Drying is one of the most widely used methods for fruit preservation. Sun drying is the most common method, because it uses a natural resource/source of heat: sunlight [Doymaz and Ismail 2010]. But sun drying is not possible as a drying method for this fruit, because *Arbutus unedo* appears in clusters and ripens in the autumn and winter months. Therefore, other drying techniques are needed for drying of *Arbutus unedo* fruits and it is known that vacuum drying may allow to obtain high quality products [Muthukumaran et al. 2008]. Generally, in fruits, drying is controlled by wax cuticle in the fruit skin and the drying rate can be increased by means of removing the surface of fruits and by chemical pre-treatments, such as ethyl oleate or potassium carbonate [Doymaz 2007]. Ethyl oleate has been commercially used to accelerate the drying and it is applied to the surface of the fruit by dipping, resulting in a coating which apparently breaks down the waxy cuticular on fruit surface, and this way the resistance to moisture loss is reduced in fruit skin [Williams 1989]. Blanching is also one of the most widely used pre-treatment because of inactivation of enzymes, and it changes the tissue structure, which results in shortening the drying time and in increasing the drying rate [Doymaz 2008].

The antioxidants are vital substances which possess the ability to protect the body from possible harms caused by the free radical [Percival 1998]. Hence, there has been an increasing interest in different medicinal and dietary plants and their antioxidant potential. During the drying process, it is well known that numerous physical, chemical and nutritional changes occur in the fruits, which can affect their quality attributes [Di Scala and Crapiste 2008]. This research was performed to determine the changes in antioxidant activity, as well as nutritional characteristics and colour properties of vacuum dried strawberry tree (*Arbutus unedo* L.) fruit and the effects of applying ethyl oleate and water blanching pretreatments.

MATERIAL AND METHODS

Material. Ripe fresh strawberry tree (*Arbutus unedo* L.) fruits were collected from Turkey Çanakkale province, Lapseki subprovince and Şevketiye village at 100-200 m above sea level.

Drying experiments. Strawberry tree fruits were treated by two different applications in order to increase the water permeability of berries. In first pretreatment, whole berries were dipped into 2% ethyl oleate + 4% potassium carbonate solution [Doymaz 2008] for one minute at room temperature (Ethyl oleate application; EO). In second pretreatment, whole berries were dipped in hot water (80°C) for one minute. The temperature was determined to be 80°C after various blanching pretreatments, because the texture of berries remained tough at this temperature (Water blanching application; WB). Control samples with non pre-treatment (NPT) were also prepared for compari-

son. Before the drying process, all berries were transversely divided into two halves. Drying experiments of samples were carried out at the drying temperature of 65°C and at the vacuum chamber pressure of 10 kPa by using the lab-scale vacuum dryer (MMM Medcenter Vacucell 22 Blue Line Vacuum Dryer). A batch of 300 g sample was placed on a tray and arranged in one single layer.

Drying processes for every treatment (NPT, EO and WB) were continued to reach approximately the same moisture content (10% w.b.). The wet-basis moisture content which is expressed as the ratio of moisture mass to the total mass of the substance, were calculated during the drying experiments. The ratio is represented by the following equation:

$$MC_{wb} = \frac{m_{water}}{m_{water} + m_{dm}} \cdot 100$$

In this equation MC_{wb} is wet-basis moisture content in %, m_{water} is mass of moisture in kg, and m_{dm} is mass of dry matter in kg.

Preparation of sample extract. For ethanol extraction of dried fruits, 5 g of ground samples were mixed with 250 mL of ethanol and the mixture was shaken by using Edmund Buhler-KS-15 shaker (at 180 rpm constant speed) for one night at room temperature. Ethanol solution was filtered through Whatman No. 4 filter and the residue was reextracted twice and filtered. All extracts were pooled and solvents were removed by using a rotary evaporator (IKA-Werke GmbH and Co. KG) under vacuum at 45°C. To determine the fresh fruit antioxidant activity properties, lyophilised samples were used for ethanol extraction. For lyophilisation, berries divided in 4 pieces were frozen by blast and fluid bed freezer (Armfield; FT 36) at -40°C immediately, later crushed and lyophilised by vacuum freeze drier (plate temperature at 10°C) and after then extracted by ethanol using the same procedure.

Total phenolic content (TPC). Total phenolic content in the extracts was determined by Folin-Ciocalteu reagent of Slinkard and Singleton [1977] method using gallic acid as a standard phenolic compound at a range between 50 and 500 µg/mL ($r^2 = 0.9897$). The amount of total phenolic compounds was expressed as mg of gallic acid equivalent (GAE).

DPPH radical scavenging activity. To determine the DPPH radical scavenging activity, the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazil (DPPH[•]) using the Blois method [1958] by using a spectrophotometer (Shimadzu UV-1601) at 517 nm. The antiradical activity was calculated using the ratio: $(A_{control} - A_{sample} / A_{control}) \times 100$, where $A_{control}$ is the absorption of the DPPH[•] solution and A_{sample} is the absorption of the DPPH[•] solution after the addition of the sample.

Total antioxidant assay using β-carotene bleaching method. Total antioxidant activity by using β-carotene bleaching method was carried out by the spectrophotometric method of Miller [1971] based on the ability to decrease the oxidative bleaching of β-carotene in a β-carotene/linoleic acid emulsion and the absorbance of the mixtures was read at 490 nm. Total antioxidant activity (TAA) was expressed as the percentage of inhibition relative to the control after a 120 min incubation period [Al-Saikhan et al. 1995], as below: $TAA = 100(DR_C - DR_S)/DR_C$, where: DR_C – degradation rate of control = $\ln(a/b)/120$, DR_S – degradation rate of sample = $\ln(a/b)/120$, a and b – absorbance of samples and controls at 0 and 120 min.

Other analysis methods. Dry matter content of *Arbutus unedo* berries was determined by using a vacuum dryer at 70°C [Cemeroglu 2007]. Ascorbic acid content (AA) of samples was analysed according to the method described by Cemeroglu [2007], which is by using 2,6-dichlorophenolindophenol solution as indicator and the absorbance was measured at 500 nm on a Hitachi 2000 UV/Vis spectrophotometer. The reducing sugar content of berries was assayed according to Ross [1959] by using 2,4-dinitrophenol reactive and glucose as a standard. The measurements were made at 600 nm by a spectrophotometer (Hitachi UV/Vis –2000). The 5-hydroxymethylfurfural (HMF) content was determined quantitatively following the procedure described by Cemeroglu [2007], which is based on the colorimetric reaction between barbituric acid, p-toluidine and HMF, forming a red colour complex. Mineral contents in samples were determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES) with a microwave burning unit (30 min at 200 PSI, 2008C, 1,200 W) [Skujins 1998]. The working conditions of ICP-AES (Varian-Vista) were the following; RF power 1.5 kW for axial, Plasma gas flow rate (Ar) 17 L/min (radial), Auxiliary gas flow rate (Ar) 0.2, Viewing height 15 mm, Copy and reading time 5-10s (max. 60 s). Colour measurements of the samples were performed by using Hunter-Lab tristimulus colorimeter (D25LT model). CIE L*, a*, b* colour parameters and yellowness index (YI) of samples were measured from 10 points of every sample pile just after drying processes. Colour parameters were measured either for exterior (skin) or inner fleshy part. In the CIE L*, a*, b* colour system L* value represented the lightness of colour (0 – black, 100 – white), a* represented the redness (positive values) or greenness (negative values) colour, and b* represented the yellowness (positive values) and blueness (negative values) colour [Hunter... 2006].

Statistical analysis. Statistical analysis was carried out by the method given by Steel and Torrie [1960], which is by using the MSTAT packaged program.

RESULTS AND DISCUSSIONS

As shown in Figure 1, the drying time was shortened approximately for 3 hours by using the ethyl oleate pretreatment. Water blanching also shortened the drying time, but the effectiveness of this pretreatment was found to be lower than that of the ethyl oleate application. In different drying studies, some chemical applications with ethyl oleate gave better results for different materials. The short drying time of the vacuum dried strawberry tree fruits using the EO pre-treatment can be explained by the fact that this solution removes the waxy layer from the surface of berry and increases the skin permeability similar to its effects on other fruits, such as strawberry, seedless grapes and apricots [Doymaz 2008].

The reducing sugar content of fresh fruit increased approximately two times in dried berries due to the moisture loss. On the other hand, in dry matter the reducing sugar content of the pretreated berries show statistically significant ($P < 0.05$) losses. The results show that WB application caused higher sugar losses than those of EO pretreatments. This higher loss can be explained by the fact that a higher temperature of water and an increase in the solubility may increase the reducing sugar content. The lowest value of reducing sugar decrease was determined also in NPT samples, and from these results can be concluded that the sugar content of dried berries was affected by pretreatments rather than by drying (Table 1).

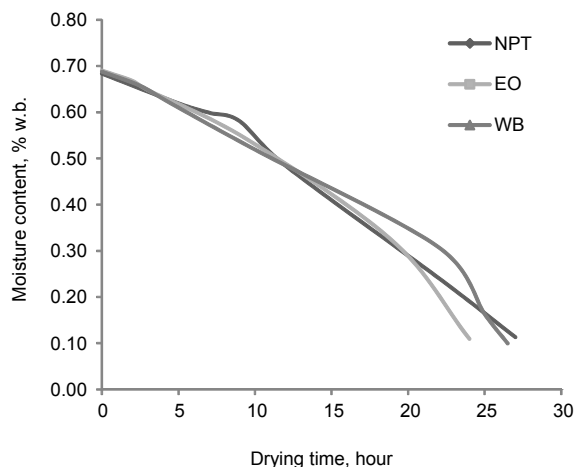


Fig. 1. Decreasing of moisture content of strawberry tree (*Arbutus unedo* L.) fruit in vacuum drying process at 65°C

Table 1. Effect of pretreatments and vacuum drying process on dry matter, ascorbic acid, total phenolic and reducing sugar content of strawberry tree (*Arbutus unedo* L.) fruit and HMF formation

Analysed samples	Dry matter content g/100 g	Ascorbic acid mg/100 g	Ascorbic acid in dry matter* mg/100 g DM	Retention of ascorbic acid in dried fruit, %	Retention of ascorbic acid in dry matter of sample, %	Extract yield g/100 g of fruit %	Total phenolic content µg GAE/g extract	Total phenolic content in dry matter, µg GAE/g	Reducing sugar g/100 g	Reducing sugar in dry matter g/100 g DM	Retention of reducing sugar in dry matter of sample, %	HMF, mg/kg
Fresh fruit	43.84 ±1.07 ^b	231.66 ±3.52 ^a	528.42 ±2.29 ^a	nd	nd	70.34 ±1.42 ^a	14.29 ±0.71 ^a	10.06 ±0.68 ^a	9.97 ±0.53 ^b	22.74 ±0.63 ^a	nd	nd
NPT	88.68 ±1.11 ^a	144.90 ±2.33 ^b	164.39 ±1.72 ^b	37.45	68.88	59.34 ±1.84 ^c	2.62 ±0.42 ^c	1.26 ±0.52 ^d	19.85 ±0.09 ^a	22.38 ±0.60 ^{ab}	1.58	2.49 ±0.56 ^a
EO	89.06 ±1.51 ^a	134.20 ±3.04 ^b	150.68 ±0.76 ^b	42.07	71.49	63.49 ±1.23 ^b	3.61 ±0.41 ^c	2.30 ±0.44 ^b	19.53 ±0.09 ^a	21.92 ±0.65 ^c	3.61	1.45 ±0.86 ^c
WB	90.03 ±1.14 ^a	124.40 ±1.96 ^c	138.18 ±1.55 ^c	46.30	73.85	57.09 ±1.54 ^d	4.53 ±0.41 ^b	2.35 ±0.44 ^b	18.78 ±0.41 ^a	20.86 ±0.85 ^d	8.26	2.25 ±0.26 ^b
LSD %5	2.093	6.002	15.825			1.075	0.532	0.047	1.797	0.511		0.006

*To determine the effectiveness of drying method on fruits, analysed values were expressed in dry weight basis.
 NPT – non pre-treated dried fruits, EO – ethyl oleate applied dried fruits, WB – water blanching applied dried fruits.
 Different letters of upper index within the column indicate significant differences at P < 0.05 level (n = 3).

Colour parameters of L^* , a^* , b^* and YI for fruits were evaluated from external and internal side in dried fruits, because the colour of fruit is red on external side and deep yellow on internal side (Fig. 2 a and 2 b). Proliac and Raynaud [1981] reported that carotenoids may be responsible for yellow colour in the flesh of the fruits, and that the external red colour is mainly due to the presence of other phenolic pigments, identified as 3-glucosylcyanidin. As seen in Figs 2 a and 2 b, the decrease on redness (a^*) in internal side was not affected by drying and pretreatments, and differences were found to be insignificant ($P < 0.05$). The decrease of the yellowness (b^*) of internal side was found significant and pre-treatments demonstrated effectiveness on saving the yellowness. Therefore, it can be concluded that the decrease of carotenoids in fleshy part of fruit was significantly affected by the drying process. Similarly, the drying process significantly effected the decrease of the external red colour, but the effect of pretreatments was found to be insignificant. On the external side, the yellowness (b^*) also decreased significantly after drying process, but EO pre-treated samples were found to be of higher yellowness than the NPT samples (Fig. 2 a). The brightness (L^*) of the dried fruits also decreased both on external and internal sides and they were differently affected by EO and WB pretreatments. It is shown that the decrease in internal side brightness was higher than in external side brightness. As seen in Figs 2 a and 2 b, the differences between the degrees of yellowness change, namely the yellowness index (YI) of both, the skin side and the inside part of strawberry tree fruits, and after drying were found statistically insignificant. In terms of pretreatments, it can be seen that EO saved the brightness of external colour higher than WB. In general evaluation, the external colour of fruits was found to be better in EO pretreatment in terms of brightness and yellowness. Ergunes and Tarhan [2006] found that all the pretreatments using ethyl oleate significantly accelerated the drying process, but the using of only 2% ethyl oleate decreased all colour parameters, while using the solution that contains 2% NaOH increased the all colour parameters remarkable. The future research should examine the effects of EO solution, including NaOH, on colour of fruit.

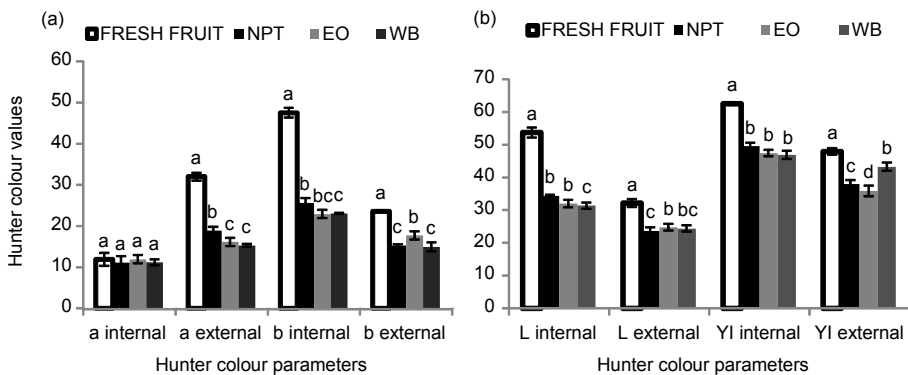


Fig. 2. Hunter colour parameters (a^* , b^* , L and YI values) of fresh and dried strawberry tree (*Arbutus unedo* L.) fruit. Values were determined both external and internal side of fruit. Different letters of upper index within the column indicate significant differences at $P < 0.05$ level ($n = 3$): $LSD_{ai} = 2.010$, $LSD_{ac} = 2.140$, $LSD_{bi} = 2.975$, $LSD_{bc} = 1.047$, $LSD_{Li} = 0.947$, $LSD_{Le} = 0.891$, $LSD_{YLi} = 8.023$, $LSD_{YLe} = nd$

The degree of browning is the most common method for brown pigment detection [Labuza and Baiser 1992], while the formation of 5-hydroxymethylfurfural (HMF) has been widely used as an indicator. In Table 1 it is shown that EO pretreatment gave better results than other applications. It could be a result of the berries being exposed to a shorter heat time in EO application. It can be concluded that the EO pretreatment may inhibit the formation of HMF during the drying

Ascorbic acid (AA) retention can be used as a quality index of dried products, because, if ascorbic acid content is well retained, the other nutrients are also generally preserved [Shitanda and Wanjala 2006]. The initial content of ascorbic acid in fresh berries decreased significantly (Table 1). There were no significant differences found in AA content between NPT and EO pretreated berries. The WB application caused the higher degradation of AA, and AA loss was found to be 46.30% in dried fruit. The berries dipped in solution in both pretreatments indicated that the losses can arise due to the higher temperature of solution by WB pretreatments. According to our results, it can be said that AA retention occurs significantly in fruits by drying process and pretreatments.

In the drying process, the protection of phenolic content is a very important issue because of the known antioxidant activity of the phenolic compounds. After the drying process, the total phenolic content of berries decreased significantly and the pretreated samples exhibited a higher phenolic content than the NPT fruits. Although the phenolic content was determined to be higher in WB treatment than in EO, there were no statistically significant differences in dry matter content (Table 1). In a previous study, Wen et al. [2010] explained that blanching process had different effects on different vegetables, and while some vegetables showed increased phenolic content, others revealed a decreasing phenolic content.

The antioxidant activity of fresh and dried berry ethanolic extracts were determined according to the free radical-scavenging activity (DPPH) and β -carotene bleaching assay methods. DPPH method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH [Shon et al. 2003]. As it can be seen from Figure 3, the inhibition effects of extracts

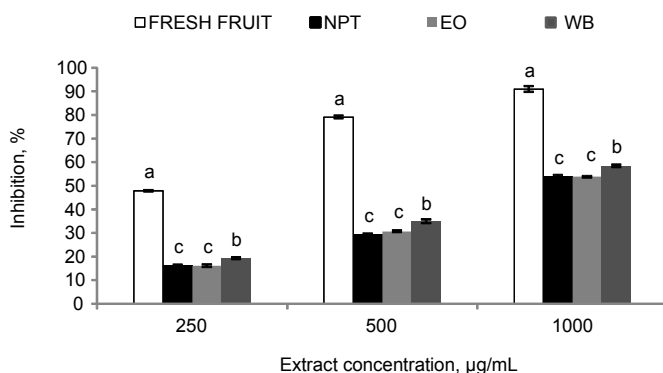


Fig. 3. DPPH Radical-scavenging activity in the ethanolic extracts of fresh and dried strawberry tree (*Arbutus unedo* L.) fruit. Different letters of upper index within the column indicate significant differences at $P < 0.05$ level: $LSD_{250} = 0.725$, $LSD_{500} = 0.886$, $LSD_{1000} = 0.99$, $n = 3$

increased with increasing concentration and the DPPH scavenging activity of fresh fruit was determined to be $91.03 \pm 1.18\%$ at $1000 \mu\text{g/mL}$ concentration. On the other hand, the scavenging activity decreased significantly after the drying process, and it was found to be $54.13 \pm 0.64\%$ in NPT fruits. In terms of pretreatment, WB application resulted in a slightly higher scavenging activity, because of the including of higher phenolic contents of extracts. The EC_{50} value is the effective concentration which is required to decrease the initial DPPH^{*} concentration by 50% and lower EC_{50} value reflects better protective action. Lower EC_{50} value corresponds to the highest scavenging activity of DPPH radicals. The lowest EC_{50} values were found in fresh berry extracts as 1.841 mg/mL , and it was 5.992 mg/mL for WB, 6.627 mg/mL for EO and 7.814 mg/mL for NPT.

The reduction in absorbance of β -carotene–linoleate emulsion in the presence of the ethanol extracts of fresh and dried berries is shown in Figure 4. In the β -carotene–linoleate system, the inhibition effects of pre-treated fruits were determined to be lower than those of fresh berries at all concentrations. The lowest inhibition effect of β -carotene bleaching was determined in NPT extract. The pretreated fruits exhibited a higher activity than NPT, and EO pre-treatment was found that had higher affect on activity. This case may be interest to apolar character of EO in the apolar reaction medium which carried out β -carotene bleaching assay. If antioxidant activity was expressed according to β -carotene bleaching system, EO pre-treated fruits were found to have a higher activity. It may explain why lower drying time causes the lower degradation of active phytochemicals.

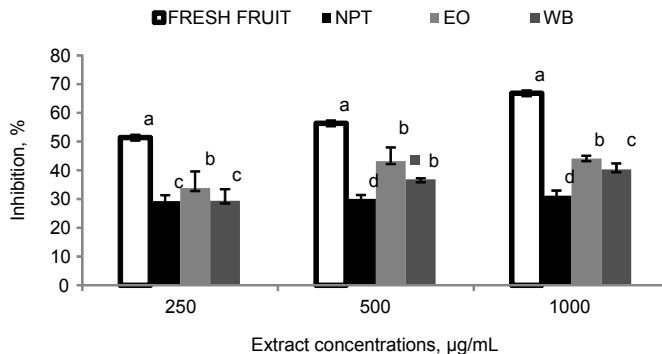


Fig. 4. β -carotene bleaching activity of ethanolic extracts of fresh and dried strawberry tree (*Arbutus unedo* L.) fruit. Different letters of upper index within the column indicate significant differences at $P < 0.05$ level ($n = 3$): $LSD_{250} = 9.372$, $LSD_{500} = 5.53$, $LSD_{1000} = 7.380$

The mineral contents of the fresh and vacuum dried berries are given in Figs 5 a and 5 b. As shown in these figures, Ca, K, and P are the predominant minerals in fresh and dried samples. The mean values of these minerals in fresh berries were determined as $645.55 \pm 90.98 \text{ mg/kg FW}$, $2931.53 \pm 467.24 \text{ mg/kg FW}$ and $492.92 \pm 69.69 \text{ mg/kg FW}$, respectively. The mineral contents was expressed in dry matter in fresh and dried fruit and exhibited in related figures to compare the drying method effects on mineral contents

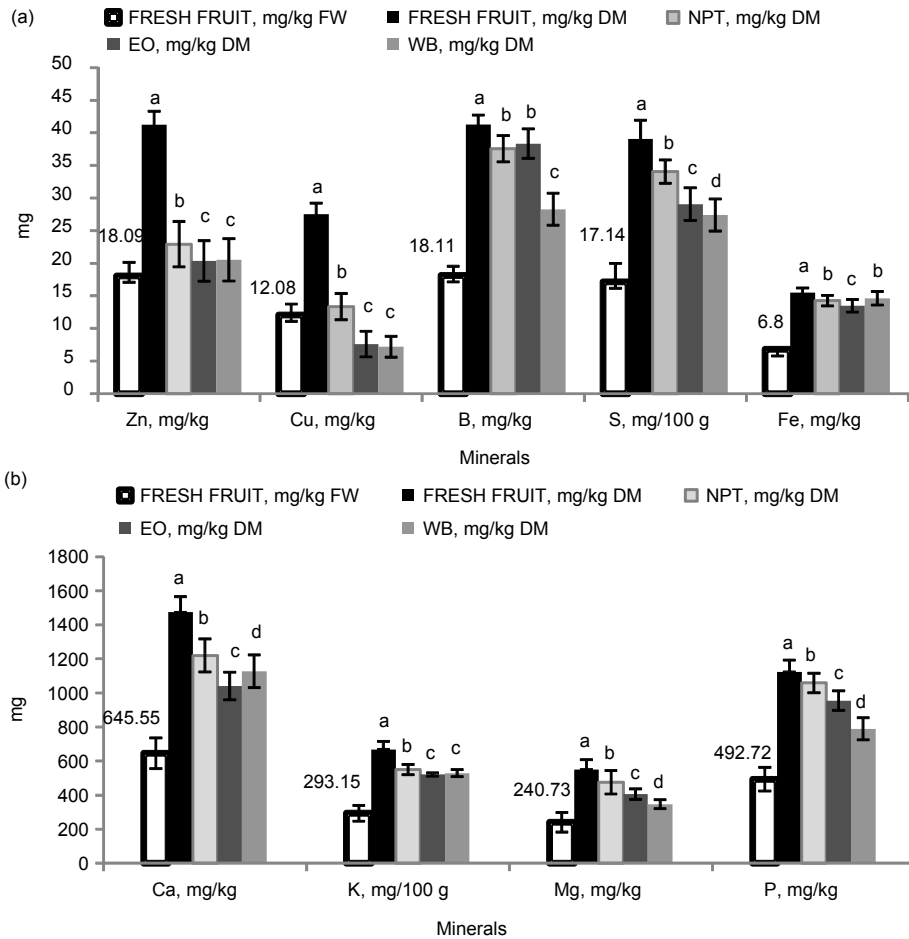


Fig. 5. Mineral matter content of fresh and dried strawberry tree (*Arbutus unedo* L.) fruits. Amount of mineral content values for fresh fruit were given in both column. First column shows amount of mineral content in fresh weight of kg (mg/kg FW) while second column shows amount of mineral content in dry matter of kg (mg/kg DM) fruit. Different letters of upper index within the column indicate significant differences at $P < 0.05$ level ($n = 3$): $Zn_{LSD} = 1.968$, $Cu_{LSDa} = 0.405$, $Fe_{LSD} = 0.701$, $Ca_{LSD} = 2.250$, $K_{LSD} = 10.069$, $Mg_{LSD} = 4.984$, $P_{LSD} = 2.310$, $S_{LSD} = 5.484$, $B_{LSD} = 1.796$

(Figs 5 a and 5 b). As it can be seen from these figures, the Fe, Ca, K and P contents in the dried berries are approximately twice higher than in the fresh samples wet weight due to the increasing of the dry matter content, and these results confirm Arslan and Ozcan [2010]. The reduction in mineral content by drying were determined to be important statistically ($P < 0.05$). Zn and Cu showed a more considerable decrease than other minerals in drying fruit with respect to the fresh samples. Additionally, Cu, K, Mg, P, S and B contents of dried berries were found to be lower in WB pre-treated samples than in the EO. WB pre-treatment increases the losses of these minerals. In EO

application, although the berries were dipped in solution, the WB process may increase the amount of mineral losses, because of the higher temperature of water in the WB pre-treatment which could cause an increase in the solubility of the elements more than the EO pre-treatment. In general, it can be concluded that the water blanching process further increased the loss of minerals, except Ca. The Ca value was found to be lower in EO pre-treated samples and this decrease may be a result of resolution of the Ca when solving the waxy substances from external surface at the same time. The level of K was high in all samples, which can be considered to be an important indicator due to the fact that many enzymes use them as cofactors [Ozcan and Haciseferogullari 2007].

CONCLUSIONS

In conclusion, the vacuum drying process shows significant effects on the colour, antioxidant activity and nutritional characteristics of strawberry tree fruit. In dried fruits, the reducing sugar content and the minerals content were found to be higher than in fresh berries, because of the increased dry matter content. EO pre-treatments shortened the drying time and some fruit characteristics were affected positively by using this pretreatment. Therefore, the strawberry tree fruit is usable in food industry with its preservation by drying method and due to the rich nutritional composition, antioxidant activity and attractive colour. The future studies should focus on a better protection of the antioxidant potential and nutritional composition of fruit by using different drying methods and pretreatments.

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WŁAŚCIWOŚCI ANTYUTLENIAJĄCE, BARWA I WŁAŚCIWOŚCI ŻYWIENIOWE LIOFILIZOWANYCH OWOCÓW CHRÓSCINY JAGODNEJ (*ARBUTUS UNEDO* L.)

Wstęp. Chociaż owoce chrósciny jagodnej zawierają więcej substancji odżywczych oraz substancji bioaktywnych niż wiele innych owoców, obecnie nie jest powszechne ich spożycie. W przedstawionej pracy badano wpływ bioliofilizacji na zmiany w wartości żywieniowej, barwie oraz zawartości substancji bioaktywnych zawartych w tych owocach.

Material i metody. Owoce były zbierane w Turcji w prowincji Çanakkale. Po wstępnej obróbce (blanszowanie oraz dodatek oleinianu etylu) były poddane liofilizacji. Oznaczono zawartość: kwasu askorbinowego, cukrów redukujących, soli mineralnych, związków fenolowych, aktywność DPPH, powstawanie HMF oraz zmiany barwy.

Wyniki. Wstępne działanie EO skracало czas suszenia bardziej niż WB i wpływało na zachowanie większej wartości aktywności β-karotenowej, mniejszej wartości HMF oraz większych wartości jasności i tonu żółtego w barwie wyrobu.

Wnioski. W pracy zbadano wpływ liofilizacji na barwę, właściwości antyutleniające oraz właściwości żywieniowe badanych owoców. Ustalono, że owoce chrósciny jagodnej mogą być wykorzystane w przetwórstwie spożywczym ze względu na ich dużą wartość żywieniową, właściwości utleniające oraz atrakcyjną barwę.

Słowa kluczowe: *Arbutus unedo* L., chróstcina jagodna, liofilizacja, DPPH, kwas askorbinowy, sole mineralne, barwa, obróbka wstępna

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