

## Chemical Composition and Antioxidant Activity of Sea-Buckthorn (*Hippophae rhamnoides L.*) Grown in Issyk-Kul Region, Kyrgyz Republic

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### ABSTRACT

The fruit of sea-buckthorn (*Hippophae rhamnoides L.*) is a rich source of nutrients and phytochemicals. This study determined the chemical composition and antioxidant activity of sea-buckthorn grown in Issyk-Kul region, Kyrgyz Republic, during maturation from August to November. Three different assays [ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity (DPPH), and cupric ion reducing antioxidant capacity (CUPRAC)] were used to determine the antioxidant activity of sea-buckthorn fruits. Sea-buckthorn fruits had a sugar content of 6.57-8.08 g/100g, a dietary fiber content of 0.39-0.83 g/100g, an ash content of 0.49-0.99 g/100g, a lipid content of 6.56-6.98 g/100g and titratable acidity of 1.38-1.92%. The total phenolic content and total flavonoids of sea-buckthorn fruits were 408.52–886.48mg GAE/kg and 260.81-345.37mg CE/kg, respectively. Antioxidant activity values determined by FRAP, DPPH, and CUPRAC were 88.51-1041.22mg TE/kg, 1011.93-2673.22mg TE/kg, and 755.40-1232.55mg TE/kg, respectively. Total carotenoid content was 16.6-18.8 mg/kg. Results showed the relationship between the change in the color of sea-buckthorn berries and the chemical composition by the months of their ripening and the influence of weather conditions. All these results indicated that sea-buckthorn fruits could be beneficial for human nutrition while they might have a great potential to be used in a wide range of products to enrich their functional properties.

**Keywords:** Sea-buckthorn, Antioxidant activity, Phenolic, Flavonoid, Carotenoid

### Kırgız Cumhuriyeti Issyk-Kul Bölgesinde Yetişen Yalancı İğde (*Hippophae rhamnoides L.*) Meyvelerinin Kimyasal Kompozisyonu ve Antioksidan Aktivitesi

#### ÖZ

Yalancı iğde meyvesi (*Hippophae rhamnoides L.*) zengin bir besin ve fitokimyasal kaynağıdır. Bu çalışmada, Kırgız Cumhuriyeti'nin Issyk-Kul bölgesinde yetişen yalancı iğde meyvelerinin ağustos ayından kasım ayına kadar olan olgunlaşma döneminde kimyasal bileşimi ve antioksidan aktivitesi belirlenmiştir. Yalancı iğde meyvelerinin antioksidan aktivitesinin belirlenmesinde üç farklı yöntem [ferrik indirgeyici antioksidan güç (FRAP), DPPH radikal süpürücü aktivite (DPPH) ve kuprik iyon azaltıcı antioksidan kapasite (CUPRAC)] kullanılmıştır. Deniz iğdesi meyvelerinin şeker içeriği 6.57-8.08 g/100g, diyet lifi içeriği 0.39-0.83 g/100g, kül içeriği 0.49-0.99 g/100g, lipid içeriği 6.56-6.98 g/100g ve

titre edilebilir asitlik değeri %1.38-1.92 arasında belirlenmiştir. Yalancı iğde meyvelerinin toplam fenolik içeriği ve toplam flavonoidleri sırasıyla 408.52-886.48 mg GAE/kg ve 260.81-345.37 mg CE/kg olarak ölçülmüştür. FRAP, DPPH ve CUPRAC ile belirlenen antioksidan aktivite değerleri sırasıyla 88.51-1041.22 mg TE/kg, 1011.93-2673.22 mg TE/kg ve 755.40-1232.55 mg TE/kg olmuştur. Toplam karotenoid içeriği 16.6-18.8 mg/kg olarak ölçülmüştür. Veriler, deniz iğdesi meyvelerinin rengindeki değişim ile kimyasal bileşimi arasındaki ilişkiyi, olgunlaşma aylarına ve hava koşullarının etkisine göre göstermiştir. Tüm bu sonuçlar deniz iğdesi meyvelerinin insan beslenmesinde faydalı olabileceğini göstermekte ve ayrıca fonksiyonel özellikleri zenginleştirmek amacıyla geniş bir ürün yelpazesinde yüksek kullanım potansiyeli vardır.

**Anahtar Kelimeler:** Yalancı iğde, Antioksidan aktivite, Fenolik, Flavonoid, Karotenoid

## INTRODUCTION

Sea-buckthorn (*Hippophae rhamnoides* L.) is native to Europe and Asia and has been domesticated in many countries such as India, China, Nepal, Pakistan, Myanmar, Russia, Britain, Germany, Finland, Romania, and France [1]. Different studies were conducted on the sea-buckthorn fruits. They showed that sea-buckthorn is a good source of phytochemicals such as vitamins, carotenoids and flavonoids, sterols, and some essential amino acids. For example, fruits and seeds of plants have been reported to possess high antioxidant potential, which is attributed to hydrophilic and lipophilic compounds, including ascorbic acid, flavonoids, proanthocyanidins, and carotenoids [2, 3].

Sea-buckthorn fruits, seeds, and other parts are rich in vitamins (A, B1, B12, C, E, and K), flavonoids, lycopene, carotenoids, and phytosterols [4, 5]. These compounds are therapeutically important because of their antioxidative properties [4, 5]. Moreover, polyphenolic compounds of sea-buckthorn were reported to possess anti-inflammatory, anti-neurodegenerative, anti-mutagenic, anti-carcinogenic, and anti-diabetic effects [6]. Several factors, such as cultivar, geography, harvesting time, and processing are known to have a significant effect on the chemical

composition of sea-buckthorn. Therefore, the antioxidative activity of sea-buckthorn fruits also depends on these factors [7, 8]. There are more than 150 cultivars of sea-buckthorn worldwide, and new varieties are still being developed [9].

In the Kyrgyz Republic, sea-buckthorn mainly grows in Issyk-Kul, Jalal-Abad, and Chuy regions. Up to now, many studies have been carried out on sea-buckthorn in different areas of the world. Still, few studies were found on the chemical composition and antioxidant activity of sea-buckthorn fruits grown in the Kyrgyz Republic. Therefore, this study aimed to analyze the chemical composition and antioxidant activity of sea-buckthorn fruits collected four consecutive times from August to November from the Issyk-Kul region of Kyrgyz Republic. Sugar content, dietary fiber content, ash content, lipid content, titratable acidity, total phenolic content, total flavonoids, and total carotenoid content of samples were determined. Moreover, the antioxidant activity of fruits was tested by three different methods.

## MATERIALS and METHODS

The sea buckthorn fruits were collected from the Issyk-Kul region (Figure 1), where sea-buckthorn grows naturally (Figure 2).



Figure 1. Sampling location on the map of the Kyrgyz Republic

Mature fruits were collected in August, September, October, and November 2019. Fruits were transferred

into the laboratory on the same day of collection and stored at 4°C until analysis.



Figure 2. Sea-buckthorn fruits grew in the Issyk-Kul region

The content of phenolic, flavonoids, carotenoids, and antioxidant activity in sea-buckthorn fruits were only determined in the samples of September and October. Since these are the marketable primary season of sea-buckthorn fruits, on the other hand, all other analyses were conducted on all samples.

### Chemical Composition

#### Physicochemical Analysis of Sea-Buckthorn Fruits

The following methods carried out the determination of moisture contents, lipids, dietary fiber, ash, total sugars and acidity in the samples. Moisture and total solids content of the fruit was determined by the AOAC official method 922.10. The lipid content in sea-buckthorn fruits was determined by the AOAC 945.44, the Soxhlet method (Gerhardt EV6 All/16, Königswinter, Germany). The fiber of the samples was determined according to AOAC method 991.43, using fiber bags (Gerhardt EV 1, Königswinter, Germany). The ash content of the fruit was determined using a Nabertherm L5/S muffle furnace. Ashing was carried out at  $520\pm 5^{\circ}\text{C}$  by AOAC 940.26. Sugars in samples were determined according to AOAC 925.36 by titration method. The total titratable acidity was determined by titration with an alkali sample solution with the addition of a phenolphthalein indicator, according to AOAC 942.15 [10].

#### Preparation of Extracts

The extract used in the total phenolic content, total flavonoids, and antioxidant activity assays was prepared using aqueous ethanol (80% v/v) according to the method performed by Eyiz et al. [11]. Briefly, 2 g of sea buckthorn (homogenized for raw material and powder for dried samples) sample was made up to 20 mL with

the solvent and homogenized using an ultraturrax (IKA T25 digital, Staufen, Germany). Then, the mixture was held at 40°C under constant shaking at 150 rpm for 2 h in a water bath (ST30, Nüve, Ankara, Turkey). The extract was stored at -18°C after filtration through an analytical filter paper.

#### Determination of Total Phenolic Content and Total Flavonoids

The Folin–Ciocalteu procedure was used in the determination of the total phenolic content of sea-buckthorn. For this purpose, a mixture of extract (0,5 mL), Folin-Ciocalteu reagent (2 mL, 0.2 N), and sodium carbonate solution (2.5 mL, 7.5%) was prepared and incubated at 50°C in a water bath for 5 min. The absorbance of the reaction mixture was recorded at 760 nm. The total phenolic content of samples was calculated as mg gallic acid equivalent (GAE)/ kg fresh weight (fw) using a calibration curve [11].

To determine total flavonoids, 0,5 mL extract, 2,5 mL distilled water, and 150  $\mu\text{L}$  of  $\text{NaNO}_2$  solution (5 %) were mixed in a test tube. After mixing and holding for 5 min, 300  $\mu\text{L}$  of  $\text{AlCl}_3$  solution (10 %) was added, and the mixture was shaken. After holding for 5 additional min, 1 mL of NaOH (1 N) was added and vigorously shaken. The absorbance of the mixture was recorded at 510 nm, and total flavonoids were calculated as mg catechin equivalent (CE)/kg fw [11].

#### Determination of Total Carotenoids Content

The total carotenoids of the samples were determined by extracting carotenoids from 2 g of the sample by using 10 mL of extraction solvent [BHT (0.1 %) in methanol:acetone:hexane, 25:25:50] by homogenization at 10000 rpm for 1 min. Then a clear extract was

obtained by centrifugation at 10000 g for 10 min at 4 °C. The absorbance of the extract was recorded at 450 nm.

Total carotenoids were calculated using the following equation.

$$\text{Total carotenoids (mg/kg fw)} = \frac{\text{Abs} \times \text{Dilution factor}}{0.25} \quad (1)$$

### Determination of Antioxidant Activity

The antioxidant activity of sea-buckthorn samples was determined by three different procedures, which are DPPH, FRAP, and CUPRAC. All these activities were performed according to the methods employed by Eyiz et al. [2020b]. Trolox® was used as the reference antioxidant in all assays, and therefore, antioxidant activities were calculated as mg Trolox equivalent antioxidant activity (TEAA)/kg fw.

### Color Analysis

Sea buckthorn fruit color values were measured using a Color Tec-PCM (HunterLab, NJ, USA) with a measurement angle of 10°, a D65 light source, and an aperture of 8 mm. by registering color coordinates L\*, a\* and b\* on the fruit surface (Joint Standard ISO/CIE, 2007). Color was determined using the CIELAB system, in which L\*, a\* and b\* denote dark-light, green-red and blue-yellow, respectively. Color values were recorded by calculating the average reflectance values measured at three different points on each fruit [12]. The results of seven repetitions per sample were averaged.

To quantify the difference in color between samples in different months, ΔE was calculated using the formula [13]:

$$\Delta E = \sqrt{(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2} \quad (2)$$

where L\*<sub>1</sub>, a\*<sub>1</sub>, b\*<sub>1</sub> are color index values for each month during maturation and L\*<sub>0</sub>, a\*<sub>0</sub> and b\*<sub>0</sub> are the reference color index values.

### Statistical Analysis

All the experiments were replicated three times, and the data were represented as mean ± SD. Comparison of mean values of three samples was analysed by the Excel program from the Microsoft Office program package using the T-test (paired sample test) with a 95% confidence interval. A p-value < 0.05 was considered statistically significant. The coefficient of variation in the samples was calculated by using the formula:

$$v = \frac{S}{\bar{c}} \times 100\% \quad (3)$$

Where:

S – standard deviation;

$\bar{c}$  – arithmetic mean for all months.

## RESULTS and DISCUSSION

### Basic Chemical Composition

The chemical properties of sea-buckthorn fruits collected at different months are given in Table 1. As can be seen in Table 1, the collection date had a significant effect on the chemical properties of sea-buckthorn fruits. While moisture content slightly increased from August to November, acidity, dietary fiber, ash, and sugar content decreased.

Table 1. Basic chemical composition of different month's sea-buckthorn fruits\*

Chemical composition	Harvest time				Mean	Coefficient of variation (%)
	August	September	October	November		
Moisture content (%)	86.7±0.650	87.0±0.210	87.07±0.32	87.5±0.490	87.07±0.049	0.33
Total Solid (%)	13.3±0.210	13.0±0.071	12.93±0.021	12.5±0.350	12.93±0.210	0.57
Acidity (%)	1.92±0.021	1.59±0.016	1.52±0.021	1.38±0.010	1.60±0.049	12.46
Lipid (%)	6.80±0.021	6.76±0.021	6.98±0.021	6.56±0.016	6.77±0.150	2.20
Dietary fiber (g/100 g)	0.83±0.016	0.65±0.020	0.57±0.036	0.39±0.010	0.61±0.056	25.90
Ash (%)	0.99±0.016	0.99±0.016	0.62±0.020	0.49±0.031	0.77±0.260	28.83
Sugars (%)	8.08±0.071	7.76±0.049	7.33±0.122	6.57±0.049	7.43±0.300	7.64

\*: Data are presented as means ±SD (Standard deviation) (n=3).

Bal et al. [14] comparatively studied the chemical properties of sea-buckthorn fruits. According to their results, the highest moisture content in pulp from berries is 84.9–97.6% for the Indian sea-buckthorn, and the lowest in varieties from Pakistan 20–32%. Our research has shown that moisture content increased from August to November ( $86.7 \pm 0.65$  %– $87.5 \pm 0.49$  %), which is statistically significant ( $p < 0.05$ ).

Bal et al. [14] reported the ash content of the berries as 1.76–1.8%. On the other hand, our results were in the range of 0.49 and 0.99%. The differences between results could be related to geographical, environmental conditions and the method of determining the ash. In the fruits of sea-buckthorn, the ash content is maximum in August and decreases with climate cooling. Therefore, ecological conditions significantly affected the ash content of sea-buckthorn fruits.

A decrease from August to November was shown in the acidity content of sea-buckthorn fruits. The lower TTA (total titratable acidity) levels in fruits harvested after August may have been caused by exposure to developing fruits to low temperatures that occurred before harvest in September, October, and November. Freezing fruits during development on the bush has been reported to damage the cellular structure, resulting in metabolic disturbances leading to changes in the acidity of the fruit [15]. The TTA of sea-buckthorn grown throughout Europe and Asia has been reported to vary from 1.49 to 4.79% expressed as citric acid [16]. Sea-buckthorn berry pulp has high total lipid content, including tocopherols, tocotrienols, carotenoids, sterols, omega-3 and omega-6 fatty acids. Zakynthinos et al. [17]. Presented on the functionality of sea-buckthorn lipids and reported that sea-buckthorn might have various beneficial effects on human health. Sea-buckthorn from the Kyrgyz Republic was determined to have 6.77% lipid content on average. This content was much higher reported for sea-buckthorn grown in China and Finland, which were  $2.1 \pm 0.5\%$  and  $3.5 \pm 0.7\%$ , respectively. Both the seed ( $7.3 \pm 1.4$  to  $11.3 \pm 2.5\%$ ) and flesh ( $1.6 \pm 0.5$  to  $2.8 \pm 0.7\%$ ) of sea-buckthorn have been reported to contain high levels of lipid, and greater than 75% of the fruit lipid is contained in the flesh [18]. The variation in lipid content of sea-buckthorn fruits between months was negligible (Table 1). Therefore, the accumulation of lipids in sea-buckthorn fruits depends on weather conditions during the growing season.

The properties of dietary fiber and its value depend on the source and the mutual proportions of the respective fractions. Dietary fiber contains many structures with diverse physical and chemical properties and can induce physiological effects on the human body [19]. According to the results, the dietary fiber content in sea-buckthorn berries decreased from August to November (Table 1).

The total sugar content of six sea-buckthorn cultivars commonly grown in Poland was reported as 1.34–2.87 g/100 g fw [20]. The sugar content in our studies (6.57–8.08 g/100g) was relatively high. Additionally, the sugar content in berries was higher in August and decreased by November. An increase or decrease in the chemical components of the sea-buckthorn fruits selected in different months is probably related to climatic changes, such as temperature, humidity, the rainy season, and the ripening period of fruits themselves [21].

The coefficient of variation characterizes the relative dispersion of the component contents in the sea-buckthorn, which shows how much the dispersion is compared to the average value. For example, the calculation of the coefficient of variation of moisture and lipid in sea-buckthorn is content to 0.328% and 2.20%, respectively. The more the results differ among themselves, the greater the coefficient of variation (Table 1).

The highest variation coefficient was in dietary fiber 25.9% and ash 28.83%. This high coefficient of variation indicates that the content of dietary fiber and ash varies significantly more between months. Various factors can influence the difference in the content of dietary fiber and ash, for example, in the work [21] it is indicated that pruning of sea buckthorn branches affected the thickness of the peel of sea buckthorn berries, which, accordingly, can affect the content of dietary fiber. Thus, the coefficient of variation makes it possible to compare the variability of the content of the components depending on the harvest time. In this case, we could conclude that with weather changes during maturation, and the degree of ripening of sea-buckthorn, some components in sea-buckthorn fruits can vary significantly.

## Bioactive Compounds

The antioxidative properties of sea-buckthorn fruits are attributed to hydrophilic and lipophilic compounds, including phenolic compounds, ascorbic acid, flavonoids, proanthocyanidins and carotenoids [22]. Therefore, total phenolic, total flavonoids and total carotenoid content of samples collected in September and October were analyzed and the results are presented in Table 2.

The total phenolic and total flavonoid content of sea-buckthorn fruits decreased in October compared to September since October is colder than September in Kyrgyz Republic cooling may be the reason for these findings. On the other hand, total carotenoids changed slightly in October compared to September. In a previous study, Zadernowski et al. [23] reported the total phenolic content of sea-buckthorn fruits as 3570–4439 mg GAE/kg db (which corresponds 428–530 mg/kg fw). Therefore, the result of the present study was consistent with the literature.

Table 2. Total phenolics, total flavonoids and total *carotenoids* content of sea-buckthorn fruits\*

Harvest month	Total Phenolics (mg GAE/kg fw)	Total Flavonoids (mg CE/kg fw)	<i>Carotenoids</i> (mg/kg fw)
September	886.48±119.52	345.37±16.41	16.6±0.509
October	408.52±2.06	260.81±42.20	18.8±1.36

\*: Data are presented as means ±SD (Standard deviation) (n=3).

The total flavonoid contents of sea-buckthorn fruits from the Kyrgyz Republic were determined as 345.37-260.81 mg CE/kg fw. Guo et al. [24] determined the total flavonoid contents of 4 different subspecies of sea-buckthorn fruits and reported the average total flavonoids as 44.3 mg/g dw. The fruits collected in the Kyrgyz Republic had much lower total flavonoids than these species. Therefore, the geographical origin could significantly affect the flavonoids of sea-buckthorn fruits. On the other hand, the decrease in phenolics from September to October could be related to the transforming phenolics into flavonoids due to the decrease in temperature (on average September +23-25°C; October +14-16°C).

Sea-buckthorn fruits are rich in pigments and lipoproteins in membranes and the fleshy mesocarp. Carotenolipoprotein complexes are located particularly in fruit membranes where polar lipids may function as bridge compounds between the polar (protein) and non-polar (carotenoids) moieties [25]. The content of carotenoids is the main parameter of sea-buckthorn,

which gives the fruit a yellow color and is an indicator of the content of vitamins. The study showed that the total content of carotenoids in sea buckthorn from September 16.6±0.509 % to October 18.8±1.36 % (mg/kg fw) changed slightly depending on the time of collection ( $p < 0.05$ ).

### Antioxidant Activity

The antioxidant activities of sea-buckthorn fruits were determined using three different techniques. Results revealed that fruits collected in September had higher antioxidant activity than those of October (Table 3). While the differences in antioxidant activity between months were about 1.5 times in DPPH and CUPRAC methods, a much higher difference was observed in FRAP. When the bioactive compounds content and antioxidant activity results are evaluated together, it can be said that flavonoids had a much higher antioxidant activity than sea-buckthorn fruits.

Table 3. The antioxidant activity of sea-buckthorn fruits (mg TEAA/kg fw)\*

Harvest month	DPPH	FRAP	CUPRAC
September	2673.22±24.863	1041.22±33.44	1232.55±2.227
October	1011.93±29.65	88.51±4.778	755.40±27.1698

\*: Data are presented as means ±SD (Standard deviation) (n=3).

Ercisli et al. [26] reported that the antioxidant activity of sea-buckthorn fruits grown in Turkey as 80.4%-93.5% which was similar to BHT, a synthetic antioxidant. Another study reported antioxidant activity (determined by the DPPH method) of different sea-buckthorn cultivars between 1.08 and 4.67 g/kg fw [27]. Saeidi et al. [28] determined the antioxidant activity of sea-buckthorn fruits grown in Iran by the FRAP method as 248.5 mmol/kg. Therefore, the result of the present study is consistent with the literature.

### Color Analysis

The results of measuring the color of sea-buckthorn fruits at four months are presented in Table 4. Significant

differences in the color indices  $L^*$ ,  $a^*$ , and  $b^*$  between sea-buckthorn fruits were not noted ( $p > 0.05$ ), except for the  $a^*$  index of the samples. The increase in the  $a^*$  value is probably due to the suspension of the maturation process and the change in pigmentation in cold weather. The content of carotenoids reaches its maximum content when sea buckthorn berries ripen. As is known, the content of carotenoids gives pigmentation in sea buckthorn berries [21]. Accordingly, after ripening, the color change can be suspended and, due to cooling, the brightness of the berries can change, turning into a darker shade.

Table 4. Colorimetric parameters of sea-buckthorn fruits\*

Harvest month	$L^*$	$a^*$	$b^*$
August	27.88±0.424	09.60±0.424	75.67±0.848
September	29.26±0.282	10.77±0.14	78.04±0.042
October	27.30±0.212	64.50±0.14	74.66±0.042
November	27.93±0.148	88.83±0.063	75.74±0.169

\*: Data are presented as means ±SD (Standard deviation) (n=3).

The calculation results showed that between August and September,  $\Delta E$  was 31.32, and between September, October, and November were close in value to 11.44 and 11.35, respectively. Moreover, it was 12.9 between August and November. The calculated  $\Delta E$  values showed a significant difference between the months during the ripening period in August and September and the end of the fruit season in November ( $\Delta E > 6.0$ , meaning clearly visible) [29]. Probably this change is associated with a change in pigmentation with the end of fruit ripening [21].

## CONCLUSION

Sea-buckthorn fruits (*Hippophae rhamnoides* L.) are rich in biologically active ingredients with high antioxidant capacity. Our research showed that the basic chemical composition, bioactive compounds, and antioxidant activity of sea-buckthorn fruits change depending on the harvesting time of sea-buckthorn fruits. Additionally, the studied sea-buckthorn berries may be a raw material for developing functional foods and nutraceutical products rich in compounds with high biological activity. Thus, the rich chemical composition of sea-buckthorn fruits can be used in the food industry for food fortification.

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