# Determination of Acidity, Total Polyphenols Content, Calcium, Magnesium and Phosphorous in Sea Buckthorn Berries

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*In Romania the sea buckthorn (Hippophae rhamnoides) is prevalent naturally grown in hills territories, Meridional and Eastern Carpathians valleys from Moldavia and Muntenia and also in the Danube Delta. It is a valuable medicinal plant being a rich source of all nutrients and phytochemicals. This paper presents the results of analyzes performed on sea buckthorn berries collected from two geographical areas: Bacau and Buzau. The juice of sea buckthorn fruits, the calcined pulp and the calcined integral fruits were analysed. Physico-chemical analysis of integral sea buckthorn berries showed: pH values in the range 3.1-3.3, acidity expressed as malic acid in the range 1.97-2.02% and polyphenols content in the range 94.65 mg GAE/g d.w. and 78.33 mg GAE/g d.w., respectively. Macro elements as Ca, Mg and P were also determined. The amount of calcium in sea buckthorn fruit was found in the range 57.71 - 80 mg/100g d.w., magnesium in the range 30.56-35.61 mg/100g d.w., and phosphorus 5.34-20.56 mg/100g d.w.*

*Keywords: Sea buckthorn fruits, acidity, macro elements, total polyphenols*

Sea buckthorn is a kind of important crop with wide popularity across the world due to its medicinal and pharmacological importance and wide occurrence.

The sea buckthorn (*Hyppophae rhamnoides*) is a fruit-bearing shrub occurring naturally in Romanian flora. It grows spontaneously in the Subcarpathian of Moldavia and Muntenia, from upper basin of the Siret river up to Olt river. In Moldova Subcarpathians the sea buckthorn is found on the valleys of Bistrita, Trotus, Putna and Milcov rivers. In the Subcarpathian from Buzau county these buckthorn shrubs have a frequency higher than in other parts of Romania. In Muntenia, the sea buckthorn can be found on the valleys of Teleajen and Dâmbovita rivers and also on the Danube Delta.

The fruit is a pseudo berry or false stone fruit, being small, oval-shaped, fleshy and 0.7-0.8 cm in length.

The pulp of fruits may be yellow or orange; it is very juicy and has a pleasant, aromatic smell. Sea buckthorn fruits are among the most nutritious of all berries and have medicinal properties being extensively exploited for treatment of sluggish digestion, stomach malfunctioning, thrombosis, hepatic injury, tendon and ligament injuries, ulcer and cancer. Many of the claims associated with sea buckthorn are related to high nutritive value in terms of vitamins, carbohydrates, macro and micronutrient elements [1].

The juice is very high in vitamins especially in ascorbic acid (vitamin C) and carotenoids. The ascorbic acid concentration ranges from 30 mg/100g to 300 mg/100g of berries in the European subspecies rhamnoides [2], from 40 to 340 mg/100g of berries in Russian cultivars belonging to subspecies mongolica, from 460 to 1400 mg/100g of berries for subspecies fluviatilis [3] and from 250 to 2500 mg/100g of berries in China [4, 5]. The juice has a variable vitamin C content, depending on temperature of ambient [4], different harvesting time, origin [6] and processing [7].

The use of some naturally occurring antioxidant molecules in foods as well as preventive and therapeutic medicine is gaining popularity. A high content of natural antioxidants including ascorbic acid, tocopherols, carotenoids and polyphenols has been detected in sea buckthorn berries [8, 9].

A large number of bioactive substances like flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glucoside compounds), organic acids (malic acid and oxalic acid), sterols and some essential amino acids also occur in sea buckthorn fruits [10].

Sea buckthorn is recommended in avitaminose states as vitamin complex (C, B1, B2, PP) folic acid, carotenes, oil, fitosterol, minerals, etc. [11].

This paper presents studies on the content in macro elements as calcium, magnesium and phosphorus and total polyphenols content of the sea buckthorn (*Hyppophae rhamnoides*).

The objectives of this work were to characterize freshly juice (J) from squeezed sea buckthorn berries (*p*H, total acidity, salinity and total content of polyphenols) and to quantify the calcium, magnesium and phosphorous content on the sea buckthorn as fruits juice (J), calcined whole pulp of fruits (P) as well as the calcined integral fruits (F).

## Experimental part

Sea buckthorn berries were harvested on the end of September of 2013 from two geographical counties of Romania: Bacau and Buzau.

An electronic balance type ABJ 220-4M (readibility 0.1 mg) KERN&Sohn GmbH was used for weighing samples. Measurement of physico-chemical parameters (pH and salinity) was done with the multiparameter Consort C – 862 device. The *p*H value adjustment on samples involved in calcium titration with EDTA was made using a Beckman pH-meter. For the all volumetric titrations a 5 mL microburette was used.

For sample calcinations we used a Nabertherm type oven with range of temperature (30-3000°C). For centrifugation of buckthorn colloidal juice we used a Universal 32 Hettich Zentrifugen centrifuge.

UV-Vis spectra were recorded and processed with the Double Beam UVD-3200 spectrophotometer, Lobomed.

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All chemical reagents used were of analytical grade. Double-distilled water was also used for preparation of aqueous solutions.

*Sample preparation*

In the experiments three kinds of samples were prepared: fresh juice of fruits (J), whole pulp berries calcined (P) and whole fruit calcined (F).

Juicing fresh sea buckthorn fruit pulp

Sea buckthorns berries fruits were washed with running water and then with deionized water twice. They were left to drain on a plastic sieve. The seeds were removed and thus the weighed (10 g) samples were subjected to squeezing operation. The juice was separated from the solid waste of pulp by filtration through cotton filter. The colloidal juice obtained was then centrifuged at 1000 rpm for 15 min. The clear juice was added to 50 mL volumetric flask and filled up with distilled water to the mark and homogenized.

Calcination and mineralization

Samples of either fruit pulp or of whole fruit of sea buckthorn were weighed (5-10g) and subjected to drying, combustion and calcination. Calcination was carried out in two stages: at 300 °C for 10 min and then at 500 °C for 10 min, too. White ash obtained was treated with a 5 M $HNO_3$ solution [12] after which the clear solution was added to 50 mL volumetric flask and filled up with distilled water to the mark.

*Sample analysis*

Values of *p*H, salinity and total acidity were determined in order to characterize freshly squeezed sea buckthorn fruits juices. Total acidity was measured by direct titration of a 10 mL sample with 0.1 M NaOH standardized solution and the results were expressed as malic acid.

*Total polyphenols content*

Aliquots of the juice (5 mL) were added in 50 mL calibrated flasks and were oxidized with 1 mL of Folin Ciocalteu reagent. The reaction was neutralized by the addition of 1 mL 20% $Na_2CO_3$ solution and the flask was filled up to the mark with distilled water. The mixture was incubated at room temperature for 90 min, and the UV-Vis absorbance of the resulting solution (blue color) was measured spectrophotometrically at 760 nm. Calibration plot was obtained with gallic acid and using this plot the total phenolic content was expressed as milligram gallic acid equivalents (GAE) per gram of sample.

To plot this calibration curve in the range of 0.022 – 0.15 mg GAE/L, 1 mL Folin Ciocalteu reagent 1:2 was added in 50 mL calibrated flasks to different volumes of standard gallic acid solution, then 1 mL sodium carbonate solution 20%, was introduced, the mixture was stirred and let standing 10 min at room temperature and the flask was filled up to the mark with distilled water. At the end, the mixture was homogenized and let under room temperature 80 min for the color stabilization and the absorbance was read at 760 nm.

*Quantification of calcium*

1. *The EDTA titration* - direct method for calcium dosage was performed as follows: 10 mL water was added to 2 mL of centrifuged sample and the *p*H adjusted to 12 with a 1% NaOH solution immediately before titration. 0.1 g of murexid is added together with a small amount of sodium diethyl-ditio-carbamate, and all ingredients are mixed together. The solution turns red. The solution was titrated with 0.001 M EDTA to a definite violet color.

Indirect method (Clark-Collip)

The method is based on the precipitation of calcium oxalate and titration with potassium permanganate. The Clark-Collip method consists in adding 1 mL of saturated ammonium oxalate on the measured sample and bringing back to the proper pH with about one drop of 0.1 N HCl by stirring and standing up to 1 h. The mixture was centrifuged and washed once, after that the precipitate was solubilized by $H_2SO_4$ concentrated solution and titrated with standardized 0.01 N $KMnO_4$ solution [13].

*Quantification of magnesium*

*The EDTA titration* method for magnesium dosage was as follows: 10 mL of water is added to 2 mL of centrifuged sample and the pH was adjusted to 9 with a buffer solution ($NH_4OH$, $NH_4Cl$). The *p*H adjustment was made using a Beckman pH meter. 0.1 g of Black Eriocrorm T is added and all ingredients are mixed together. The solution turns violet. The solution was titrated with 0.001 M EDTA to a definite blue color.

Spectrophotometric method

The protocol of this method is described by Levine and Cummings [14]. Magnesium reacts with Black Eriocrorm T and forms magnesium chelate with red colour. The unknown and standard solutions are reading against the blank reagent at 520 nm. The linearity of the method follows the Lambert-Beer law for the concentration range 0.10-40.00μg/mL.

*Quantification of phosphorous as phosphate*

The phosphates were determined using molybdenum blue spectrophotometric method with acid ascorbic as reducing agent. A volume of 2 mL of sample with unknown concentration of phosphate, 1 mL of ammonium molybdate (50 g/L) solution and 1 mL of acid ascorbic (5 g/L) were added in a 50 mL calibrated flask. After 10 minutes it was fill up with distilled water to the mark and homogenized. The absorbance was read at 715 nm. Linearity of the method was established for the concentration range 0.04-0.24 mg/L (6 solutions) of $K_2HPO_4$, Merck phosphate certified solution. Each concentration level was determined in three repetitions.

*Statistical analysis*

Statistical analysis was used for t-test calculations of correlation coefficient (r). It was used to assess the relationship between the both kind of methods used for calcium and magnesium measurements. The results reported here are based on triplicate analyses for each sample and were expressed as the mean value ± standard deviation.

**Results and discussions**

Freshly sea buckthorn juice obtained by squeezing, filtering, and centrifugation was subjected to physico-chemical analysis in order to characterize the acidity, salinity and total content of polyphenols.

The results regarding physiochemical characteristics are shown in table 1.

Total acidity is expressed either as mL 0.1 N NaOH/L either in miliequivalents of NaOH 0.1 N/L. In the case of fresh fruit sea buckthorn juice the acidity is expressed as organic acid (g/100 mL). Among all organic acid components in sea buckthorn juice (citric acid, malic acid, oxalic acid, tartaric acid, succinic acid), the malic acid is

**Table 1**
PHYSICO-CHEMICAL CHARACTERISTICS OF FRESHLY SEA BUCKTHORN JUICE

| Sample | pH | Total acidity (malic acid g/100mL) | Salinity | Total polyphenols (GAE mg/g d.w.) |
|---|---|---|---|---|
| Freshly sea buckthorn juice from BACAU county | 2.98±0.06 | 2.929±0.023 | 2.80±0.12 | 94.65±0.65 |
| Freshly sea buckthorn juice from BUZAU county | 3.26±0.04 | 1.807±0.213 | 1.92±0.02 | 78.33±0.29 |

major acid ranging from 1.11 g/100 mL to 6.08 g/100 mL [3]. We chose expressing acidity as malic acid equivalents. The sea buckthorn juice from Buzau county has a relatively lower value of total acidity than the sea buckthorn juice from Bacau county. The values obtained are comparable to Russian genotypes (2.1-3.2 g/100 mL) results published by Zeb [3] and are similar to the ones (total acidity 2.27%, and *p*H 2.8) obtained by Lipowski [15].

Salinity expresses the amount of total dissolved salts in sea buckthorn juice. A low *p*H favors the solubilization of salts which explains the difference in salinity between the two types of sea buckthorn juice (table 1).

The determination of total polyphenols is based on the reduction of a phosphowolframate – phosphomolibdate complex to blue products by soluble polyhenolic compounds, in sodium carbonate media. The measurement of the absorption of the formed complex is performed at the wavelength of 760 nm. Equation describing the calibration curve is as follows: Y=0.4317X-0.024, $R^2$=0.09934, where Y is absorbance and X is the concentration of total polyphenols expressed as gallic acid equivalents (mg GAE/ g dry weight). The absorbance relative to a gallic acid standard curve was measured and results are shown in table 1.

The total polyphenolics content from the both fresh juices (Bacau, Buzau) is different but the difference is small (1.63%). The results are in agreement to literature [14].

From figures 1 - 4 it can be seen that the concentration of calcium and magnesium in sea buckthorn fruits harvested from Bacau is higher than fruits harvested in Buzau county.

Regarding the method of analysis applied to dosing Ca it can be stated that the Clark-Collip method provides greater concentration values with differences ranging from 4% to 12% compared to complexonometric method. Spectro-

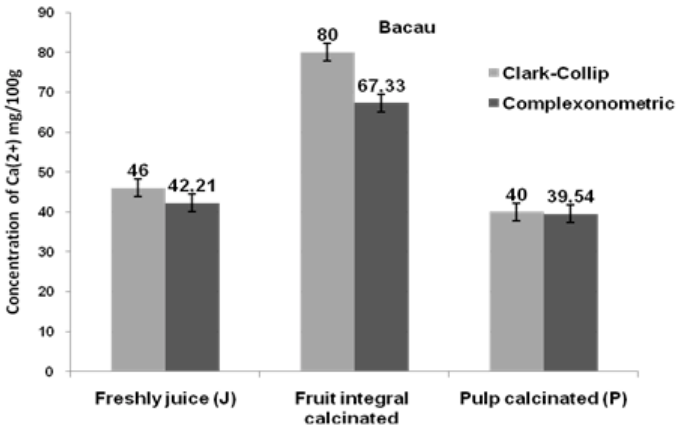


Fig. 1. The concentration of $Ca^{2+}$ on the sea buckthorn fruits juice (J), calcined whole pulp of fruits (P) and $Ca^{2+}$ calcined integral fruits (F) from Bacau county

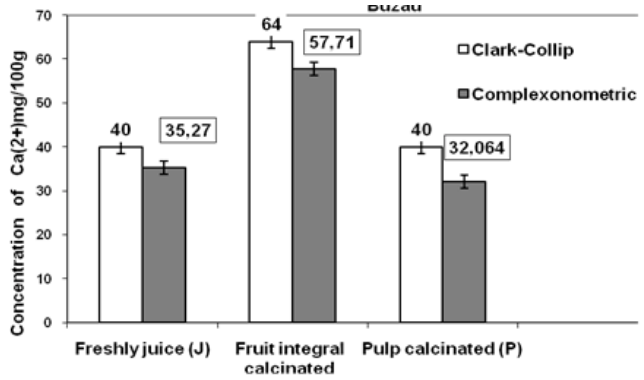


Fig. 2. The concentration of $Ca^{2+}$ on the sea buckthorn fruits juice (J), calcined whole pulp of fruits (P) and calcined integral fruits (F) from Buzau county

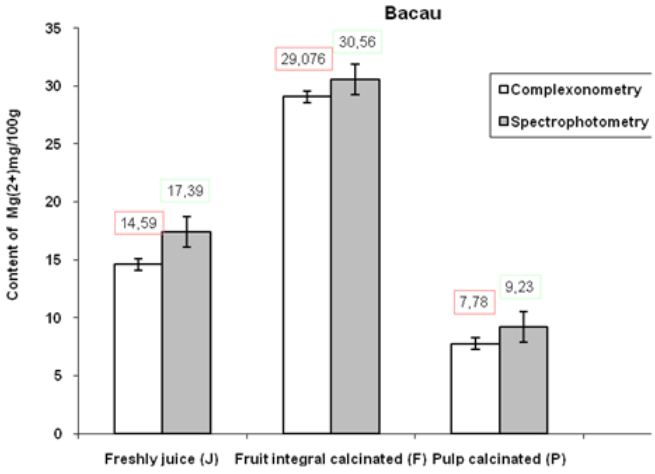


Fig. 3. The concentration of $Mg^{2+}$ on the sea buckthorn fruits juice (J), the calcined whole pulp of fruits (P) and the calcined integral fruits (F) from Bacau county.

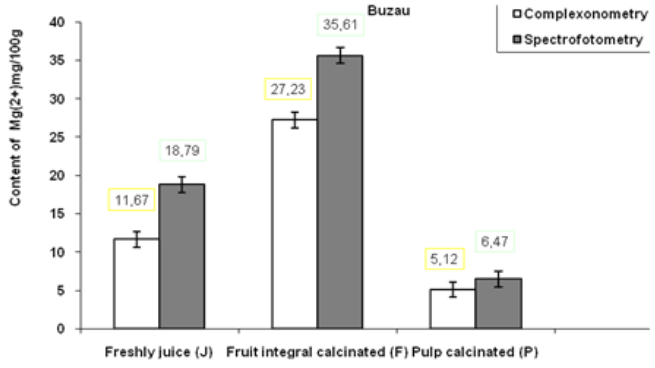


Fig. 4. The concentration of $Mg^{2+}$ on the sea buckthorn fruits juice (J), the calcined whole pulp of fruits (P) and the calcined integral fruits (F) from Buzau county

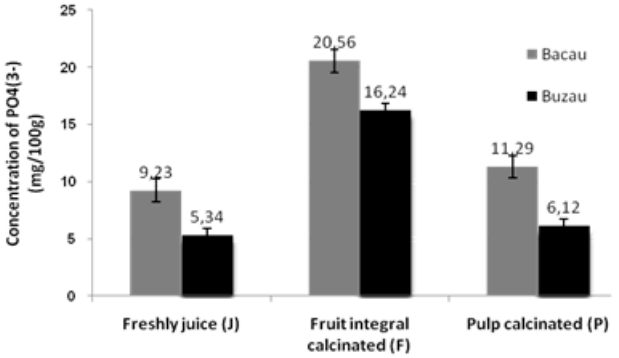


Fig. 5. The concentration of total phosphorus on the sea buckthorn fruits juice (J), calcined whole pulp of fruits (P) and calcined integral fruits (F).

photometric method for the dosage of $Mg^{2+}$ provides higher levels of Mg concentrations compared with complexonometric method, the difference between the two sets of values ranging from 1.35% to 8.38%. Results of the content of calcium and magnesium are lower compared with those found in the literature [3, 16]. One of the causes is the mineral composition of the soil but very important is especially soil reaction. A soil with low pH provides a higher content of Ca and Mg to the plant than a soil with neutral or slightly basic *p*H. The level of macro nutrients could be also affected by the fruit maturity [17].

The amount of phosphorous was quantified using molybdenum blue spectrophotometric method.

Equation of calibration curve was $Y=0.564X$, and $R^2=0.9892$, where Y is absorbance and X is the concentration of phosphate ions $PO_4^{3-}$ in mg/mL. The phosphorus concentration found in sea buckthorn fruits juice is less than in fully fruit or fruit pulp ash (fig. 5). By calcinations of samples the entire amount of phosphorus occurring in both inorganic and organic phosphorus compounds was recovered. The results are consistent with the literature [18]. Knowing that the daily intake of $Ca^{2+}$ is 350-1100 mg/day and $Mg^{2+}$ is 300-350 mg/day [19] it may recommended a diet of fresh juice of sea buckthorn every year in October.

Although fresh sea buckthorn juice tastes sour and astringent it can be mixed with honey [20] and fresh mineral water.

## Conclusions

The present study investigated the physico-chemical properties of juice from sea buckthorn fruits, pulp of fruits and integral sea buckthorn fruits with seeds. The amounts of calcium, magnesium, phosphorous occurred in the sea buckthorn show different degrees of variation from the reported literature. Also the differences between the two types of buckthorn from Bacau and Buzau counties were found which could be caused by mineral composition of the soil in which plants grow.

Sea buckthorn juice will help to fortify the body through intake of micronutrients, macronutrients, sugars, ascorbic acid and other vitamins.

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Manuscript received: 4.04.2016