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PHYSICO-CHEMICAL CHARACTERISTICS, ANTIOXIDANT ACTIVITY AND MINERAL CONTENT OF HAWTHORN FRUITS FROM SUCEAVA COUNTY

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Abstract: *The study of local hawthorn fruits is of particular interest as they often contain high amounts of bioactive compounds. The objective of this research was to determine the physico-chemical characteristics of hawthorn ripened fruits, their color using CIELab system and their antioxidant capacity by DPPH method, based on scavenging 2,2-Di (4-tert-octylphenil)-1-picrylhydrazyl (DPPH) radicals. In addition, the content in mineral elements of hawthorn fruits was determined by Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The result showed that hawthorn fruits have a pulp / kernel ratio of 1.44. The moisture, acidity, total soluble solids content, pH, pectic substances, protein, fat, ash, carbohydrates and energy values of hawthorn fruits were found as 69.14%, 0.47%, 22.20%, 5.96, 1.01%, 3.5%, 0.80%, 1.75%, 24.81% and 123.48 kcal/100g, respectively. Hawthorn fruits ripened (maturated) have a bright red color, an IC_{50} value of 53.47 µg/mL and contain high amounts of Ca (580.43 ppm), Mg (278.31 ppm) and Na (264.04 ppm). It is very important to evaluate the physico-chemical characteristics for the storage and processing of fresh fruits. This study revealed that hawthorn fruits from the local area should be considered a healthy product due to their bioactive compounds and are more suitable products than marmalade, jams, jelly, beverages and other food products.*

Keywords: *hawthorn fruits, chemical composition, colour, antioxidant activity, mineral elements*

1. Introduction

Hawthorn is a wild plant species from the rose family which includes over 280 species [1-4]. Comparatively with North America that is the center of distribution and diversity for the genus *Crataegus spp.*, in Europe and Asia there have been recognized more than 60 species [5]. *Crataegus monogyna* and *Crataegus laegivata* is the hawthorn species found in Europe, and Romania, respectively. Hawthorn has edible fruits of rounded, oblong or pear-shaped form, relatively small and range from orange-yellow, scarlet, red, yellow, blue, to black color. The flesh is mealy and dry, like those of rose hips [2]. *Crataegus monogyna* plant belongs to the *Rosaceae* family and is a small tree with thorny branches, green leaves, white flowers and bright red berries. The fruit has one woody kernel and is fully ripened in the early to mid-autumn [4; 6]. The color of ripe hawthorn fruits ranges from yellow, to green, red and dark purple [6; 7]. This is due to the constituents like carotenoids and anthocyanin and it is influenced by the maturity level [8].

Hawthorn was used in the traditional medicine of many cultures since antiquity, especially for its cardiovascular effects [9; 10]. In addition, hawthorn berries have been used as astringent, antispasmodic, diuretic, and antiatherosclerotic agents [10]. In traditional Chinese medicine, hawthorn fruits are used as a peptic agent for stimulating digestion and promoting the function of the stomach, improving blood circulation, and removing blood stasis. Beneficial effects in the treatment of indigestion with epigastric distension, diarrhea, and abdominal pain, as well as amenorrhea, hypertension, and hyperlipidemia have been reported on the fruits preparations [4]. Hawthorn extracts have been shown to increase myocardial contractility, reduce reperfusion arrhythmias, dilate peripheral arteries, and mildly decrease blood pressure [11-13]. These beneficial effects are attributed to the active constituents of hawthorn such as flavonoids and proanthocyanidins [4; 14-16].

In actual herbal medicine, hawthorn is used for its hypotensive, cardio-tonic, coronary-dilative, and anti-arrhythmic action due to the bioactive compound such as flavonoids, organic acids, procyanidins [17]. Hawthorn fruits or berries have diuretic and antidiarrheal effect [18]. Also, hawthorn fruits contain compounds with antioxidant and antilipoperoxidant activity such as epicatechin, hypersoide and chlorogenic acid.

Various studies confirmed the potential of hawthorn fruits as a good source of compounds with free radical scavenging activity [19; 20]. Some studies revealed that hawthorn berries are considered to be healthy and nutritious [21] and are consumed by children as they believe it is a good vitamin supplier [22; 23]. There are many studies that revealed the multiple health-promoting effects of hawthorn fruits on the human body.

Investigations on chemical composition of hawthorn fruits highlight that they are rich source of protein, fat, carbohydrates and mineral elements [6; 21; 24]. In addition, hawthorn fruits contain vitamins B1, B2, B6 and C, aminoacids, sugars, β-carotene, organic acids, antioxidants, and phenolic compounds such as phenolic acids, flavones, flavonols and proanthocyanidins [1; 6; 21; 25-27]. Hawthorn fruits are an excellent food source of dietary fiber and have higher pectin content than other available fruits [28]. Pectin is a widely used food additive that functions as thickener, gelling agent, and food fiber human health supplement. Dietary supplementation with pectin may reduce serum total cholesterol levels, decreases low density lipoprotein cholesterol, and moderates the glucose response [28].

Recent studies have demonstrated that hawthorn fruits are increasingly widely used as medicine and as foodstuff due to their important role in human health and nutrition. Hawthorn fruits are particularly used in herbal tea and juices production [4; 21] or to produce canned fruits, jams, jellies, alcoholic beverages and other drinks [7; 29; 30]. Accordingly, hawthorn species used in this study are abundantly grown in Romania and the fruits are becoming more popular.

The study aims to investigate some physical and chemical characteristics of hawthorn fruits. The antioxidant activity and mineral elements of hawthorn fruits were also analyzed. This study wants to underline the importance and the potential of these hawthorn berries in order to be valorized in products such as marmalade, jams or jelly.

2. Materials and methods

2.1. Materials

Wild hawthorn fruits were collected after ripening, during the harvest season in

2015 from Gura Humorului area, Suceava County, Romania. The pulp was separated from the kernel manually after the fruits were selected and washed in running water.

2.2. Methods

2.2.1. Physico-chemical analysis

The hawthorn fruits samples were analyzed to determine the physico-chemical properties according to Romanian or international standards methods: moisture content and volatile matter (SR ISO 1026:2008), total acidity (SR ISO 750:2008), soluble solids content (SR ISO 2173:2008), pH (SR ISO 1842:2008), fat content (SR EN ISO 659:2009), protein content (SR EN ISO 20483:2007), total ash content (SR ISO 763:2008). The pulp / kernel ratio was determined by gravimetric methods after pulp separation. The pectic substances content of berries was achieved by the gravimetric determination of calcium pectate according to the method described in the literature [31]. Total carbohydrates from hawthorn fruits were calculated by difference: Carbohydrates content (%) = 100 – (amount in percentage of protein, fat, moisture and ash). The t*otal energy* was calculated using the following equation: Energy (kcal/100g) = 4.1 x (g protein + g carbohydrates) + 9.3 x (g fat).

2.2.2. Colour analysis

The colour of hawthorn fruits was measured by reflectance spectroscopy using an Ocean Optics spectrometer (USA). The samples were placed in 20-mm thick holders and measured against a black and white background. Translucency was determined by applying the Kubelka–Munk theory for multiple scattering to the reflection spectra [32]. Colour coordinates were determined using the CIELab colour system where the L^* value is a measure of lightness ranging from 0 (black) to 100 (white), the a^* value ranges from -100 (greenness) to +100 (redness), and the b^* value ranges from -100 (blueness) to +100 (yellowness). The coordinates L^*, a^* and b^* were obtained from $R\infty$ between 400 and 700 nm for D65 illuminant and from 2° observer [33].

2.2.3. DPPH radical-scavening activity

The antioxidant capacity of hawthorn fruits was achieved by DPPH method, based on scavenging 2,2-Di (4-tert-octylphenil)-1-picrylhydrazyl (DPPH) radicals using a T70 UV-VIS Spectrophotometer (PG Instruments Ltd.). Free radical scavenging activity was determined according to the method described by Ercisli et al. (2015) with some modifications.Various concentrations of hawthorn fruit extract (from 10 to 240 µL) were added to freshly prepared DPPH solution (500 µL of 0.004% DPPH) in methanol, and then the mixture was left to stand for 30 min in the dark. Inhibition of the free radicals, DPPH as a percentage, was determined by measuring the absorption at 517 nm against a blank assay. Lower absorbance of the reaction mixture indicated higher free radicals scavenging activity in hawthorn fruits. The inhibition, as percentage, is calculated as the absorbance of the sample divided by that of DPPH control at the same time multiplied by 100 (Eq.1).

$$I\ (\%) = [1 - (A_1/A_o)] \times 100 \qquad (1)$$

where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of all of the extract samples and standard.

The IC_{50} value was determined by linear regression analysis using a calibration curve with different amounts of

DPPH. Antioxidant activity, expressed as the IC_{50} is the scavenging concentration ($\mu g \cdot mL^{-1}$) of the extract which quenched 50% of the initial DPPH radicals under the experimental conditions given.

2.2.4. Minerals content analysis

The mineral elements from hawthorn fruits were determined by ICP-MS technique. The method is based on ash digestion with concentrated nitric acid (65% HNO_3, Sigma Aldrich, Germany) and hydrogen peroxide (30% H_2O_2 pure p.a, Sigma Aldrich, Germany), sample dilution and ions quantitative determination using ICP-MS equipment. The element standard solutions were prepared by diluting a stock solution of 1000 mg/L of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn. All reagents were of analytical grade.

An Agilent Technologies 7500 Series system coupled plasma-mass spectrometer with a detection limit of 10^{-12} was used for efficiently detecting, identifying and reliably quantifying heavy metals and trace elements from hawthorn fruits.

The ICP-MS parameters used for analysis have been: nebulizers 0.9 mL/min, RF power 1500 W, carrier gas 0.92 L/min, mass range 7-205 uma, integration time 0.1 s, acquisition 22.7 s. Detector parameters have been: discriminator 8 mV, analogue HV 1770 V and pulse HV 1070 V.

2.2.5. Statistical analysis

All the analytical determinations were performed at least three times and the value reported for characteristics determined is the average ± the standard deviation (S.D.) value. Statistical analyses were performed on statistical software Microsoft Excel version 2003.

3. Results and discussion

3.1. Physico-chemical characteristics of hawthorn berries

The mean, maximum and minimum values, as well as standard deviations are showed in Table 1.

The average pulp / kernel ratio of hawthorn fruits varied between 1.32 and 1.55. The pulp / kernel ratio of our study was lower than those of the study carried out in some regions from Turkey [35; 36].

The moisture and volatile content of hawthorn fruits, a very important parameter when determining the physical properties of fruits, indicates a mean value of 69.14g/100g fresh weight. The moisture content found can help to suggest the stability in storage of fruits, as higher the moisture content is, more the risk of spoilage of berries. The value obtained for moisture content is close to those obtained by Özcan et al. (2005) from hawthorn fruits grown in Turkey (64.26 g/100g) or by Erfani-Moghadam et al. (2016) from hawthorn fruits grown in different geographical sites from Iran (56.88 g/100g).

The result for titratable acidity of the hawthorn fruits, calculated as 0.46% mean value is in agreement with the results reported by Türkoğlu et al. (2005) and Erfani-Moghadam et al. (2016).

The total soluble solid value was at mean value of 22.19%, while the pH values of hawthorn fruits (from 5.89 to 5.99) indicating are relatively less acidic than peach, apples and pears. In similar research on hawthorn fruits from different regions of Turkey, Balta et al. (2006), Ozcan et al. (2005) and Türkoğlu et al. (2005) reported that total soluble solid and pH values ranged from 11.66 to 24.00% and 3.12 to 4.09, respectively. The values obtained for pH are higher than those reported by previous mentioned studies.

The pectic substances estimated as calcium pectate revealed that hawthorn fruits are a good source of pectic substances, with content varying in the ranges of 1.00 - 1.03 %. A study about this complex polysaccharide revealed that the pectin pentaoligosaccharide from hawthorn was able to up-regulate the gene and protein expressions of peroxisome proliferator-activated receptor [39].

Table 1.
Chemical characteristics of hawthorn fruits

Parameter	Max.	Mean	Min.	S.D. (±)
Pulp/kernel ratio (%)	1.55	1.44	1.32	0.12
Moisture content (%)	70.13	69.14	68.00	1.07
Acidity (%, malic acid)	0.48	0.47	0.46	0.01
Total soluble solids (%)	22.90	22.20	21.23	0.86
pH	6.00	5.96	5.90	0.06
Pectic substances content (%)	1.03	1.01	1.00	0.02
Fat content (%)	0.98	0.80	0.61	0.19
Protein content (%)	3.65	3.50	3.35	0.15
Ash (%)	1.92	1.75	1.58	0.17
Total carbohydrates (%)	25.60	24.81	24.03	0.78
Energy (kcal/100g)	122.87	123.48	119.16	4.67

Fat was the less abundant macronutrient being at mean vale of 0.80g/100g dry weight (dw) for hawthorn fruits, while the protein content was found at a mean value of 3.50g/100, in agreement with the result obtained by Baros et al. (2010). The protein content was found similar to those reported by other researchers [6; 24].

Ash dried hawthorn fruits falls between proteins and fat contents, with a mean vale of 1.75g/100g. Similar values were found for hawthorn fruits from Turkey: fat 0.87%, protein 2.48% and ash 2.28% [18].

Regarding the carbohydrates content (24.81%), obtained by difference, were the most abundant macronutrient, these wild fruits can have a significant contribution to daily diets, especially during autumn and early winter.

In addition, hawthorn berries presented a high energetic value (123.48 kcal/100g) but it is higher than the value reported by Özcan et al. (2005) from Turkey hawthorn fruits (34.02 kcal/100g).

The chemical composition of hawthorn fruits is influenced by genetic factors, state of ripening, soil structure, climatic factors and other environmental factors [1].

3.2.2. The colour

The colour and appearance represent essential attributes considered by the consumer to decide the acceptability and overall quality of food [40; 41]. Anthocyanins are the phytochemicals substances which can influence the red colour of hawthorn fruits [42]. Also, the chemical composition and variety are some factors that can influence the colour of fruits.

The CIE L*a*b* values for fresh hawthorn fruits showed that the samples had a mean value of 62.12 for L*, while a* and b* were 3.30 and 3.86, respectively. The hawthorn fruits were red in colour with a slight dark red tinge, which is indicated by the positive b* value. The positive a* value with lower lightness values in hawthorn fruits shows that these fruits have darker red colour. The hue angle (Eq. 2) and chrome (Eq. 3) were calculated using the values of a* and b*.

$$h^*_{ab} = \arctan\frac{b^*}{a^*} \quad (2)$$

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

The results showed a chrome value of 5.08, while the hue angle value is 0,86, indicating the presence of different pigments in fruits, such as oligomeric procyanidins, according to result reported by Liu *et al.* (2011).

Hawthorn fruits have a red brighter colour that may be related to their richness in anthocyanins. High clearness of fruits (lightness values) can be explained by low intensity of the phenomenon of non-enzymatic browning (reactions of Maillard) due to the high content of water and the short period of storage of these samples.

The duration and the temperature of storage are some factors that influence the degradation of colour and pigment.

3.2.3. DPPH radical-scavening activity

In DPPH assay, the hawthorn fruits had the highest 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity 53.47 ($\mu g \cdot mL^{-1}$). This result revealed that the hawthorn fruits have considerable antioxidant potential that can be due to their polyphenolic compounds [25; 43]. The studied hawthorn fruits highlight a similar DPPH scavenging activity with the hawthorn berries from Serbia (52.04 ($\mu g \cdot mL^{-1}$) studied by Tadić et al. (2008).

3.2.4. Minerals content

The composition of major and trace mineral elements of the hawthorn fruits is given in Table 2. Hawthorn fruits are rich in both macro- and micronutrients, providing a nutritionally significant content of minerals.

Calcium (Ca), magnesium (Mg) and sodium (Na) were the predominant elements in the hawthorn berries pulp, followed by aluminum (Al), barium (Ba), manganese (Mn), chromium (Cr) and selenium (Se). Ca, Mg and Na were found also in rose fruits samples as the major elements [24]. Ca has an essential role in the organism; containing substances which are required by children, pregnant and lactating women for bones and teeth development [44; 45]. Mg plays an essential role in muscle relaxing along the airway to the lungs thus, allowing asthma patients to breathe easier [46]. Mn is believed to support the immune system, regulates blood sugar levels and is involved in energy production and cell reproduction [47].

Table 2.
Mineral contents of hawthorn fruits

Minerals	Values (ppm)	Minerals	Values (ppm)
Al	47.02	Li	1.08
As	0.30	Mg	278.31
Ba	11.30	Mn	8.82
Be	5.85	Na	264.04
Ca	580.43	Ni	3.29
Cr	7.08	Se	4.94
Cu	3.20	Tl	0.02
Fe	0.77	V	0.76
Ga	0.03	Zn	0.87

Also, essential elements for human body like cupper (Cu) iron (Fe) and zinc (Zn) were found in hawthorn fruits. Deficiency of Cu can cause cardiovascular disorders as well as anemia and bone and nervous systems disorders [48]. Fe is an important mineral element in the diet of pregnant women, nursing mothers, infants convulsing patients and elderly to prevent anemia and other related diseases [49]. Zn, an essential trace element for protein and nucleic acid synthesis and normal body

development, is said to be vital during periods of rapid growth and during recovery from illness [50].

Other elements with beneficial pharmacological properties that were found in hawthorn fruits are barium (Ba), nickel (Ni) and lithium (Li), which have not been established as essential. For example, Li was used in the treatment of manic depressive disorders [51].

Hawthorn fruits are a good source of macro and micronutrients which are expected to speed up metabolic processes, improve growth and development. The content of mineral elements from hawthorn fruits is influenced by various factors such as variety, growing condition, geographical origin, soil type, storage condition, climatic conditions e.g.

The analytical values of hawthorn fruits revealed the nutritional properties and mineral contents of hawthorn fruits. These results may be useful for the food industry, for many foodstuffs preparation, such as jam, jelly, etc. and for development of new products based on hawthorn fruits.

4. Conclusion

Regarding the chemical composition, the results showed that hawthorn fruits could be considered as a potential source of bioactive compounds with high antioxidant activity, having many health benefits.

Hawthorn fruits are an energetic food that may be consumed such as or could be used in the manufacture of value-added products such as jelly, marmalade, jams, wine and other drink with commercial value.

The knowledge of the chemical composition of this fruits will encourage the manufacturers to develop the production of hawthorn jelly on an industrial scale. The use of hawthorn fruits may also be attractive to consumers as a positive alternative to conventional fruits in jelly production.

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