DETERMINATION OF CHEMICAL COMPOSITION AND FATTY ACIDS OF BLACKTHORN FRUITS *(PRUNUS SPINOSA)* GROWN NEAR CLUJ-NAPOCA, NW ROMANIA

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Abstract. The aim of this study was to determine the chemical composition and the fatty acids in blackthorn fruits *(Prunus spinosa)* in order to valorize them as sources of nutrients. Different analyses, such as dry matter, protein, fatty acids, ash and mineral contents (Na, K, Ca, Mg and P) were performed. The blackthorn fruits were sampledfrom wild-grownbushes, near Cluj-Napoca, NW Romania. After sampling, the fruits were washed and freeze dried. The following analyses were investigated: (*i*) dry matter and ash contents by gravimetric methods, (*ii*) mineral content by inductively coupled plasma optical mission spectrometry (ICP-OES) after microwave-assisted acid digestion, (*iii*) protein content using Flash EA 2000 CHNS/O analyzer and (*iv*) fatty acids by gas chromatography with flame ionization detector (GC-FID). It was reported that blackthorn fruits are rich in minerals and fatty acids. In blackthorn fruits, polyunsaturated fatty acids predominated over monounsaturated fatty acids due to the abundance of oleic acid (C18:1). These fruits also contain high levels of linoleic acid and lower amounts of α-linolenic acid. The obtained results revealed good nutritional properties of blackthorn fruit. Therefore, blackthorn fruits can constitute a source for products of great pro-healthy properties(tincture, wines, and teas).

Keywords: *Prunus spinosa*, chemical composition, fatty acids

INTRODUCTION

Blackthorn (*Prunus spinosa*) is the fruit of *Prunus genus* from the Rosaceae family. Blackthorn is a thorny shrub or small tree widespread in the temperate regions of the northern region of Asia (Owczarek *et al*., 2017) and in temperate regions of Asia (Meschin *et al*., 2017). Their common names include: blackthorn, grater, rasp, sloe or stickleback and are closely related to the plums, cherries, peaches, nectarines and apricots (Kucharska *et al*., 2008; Pelc *et al*., 2010). Fully ripe blackthorn contains water (about 86%), sugars, acids, tanning substances, proteins, cellulose, tannin, polyphenols, anthocyanins, minerals (K, Ps, Fe, Cu, Na), provitamine A, vitamins B1, B2, PP, C. The consumption of minerals helps to prevent diseases (Leterme *et al*., 2006). Mineral deficiencies depend on the processing practices, the physiological and health state of the human body and the daily consumption of blackthorn (Barbera *et al*., 1992).

Each of the 130 trillion cells of the human body is delimited by one membrane, which has an extremely important role. Phospholipids and glycolipids are the most important components of the cell membranes. The physical properties of these membranes are influenced by thephospholipid fatty acids types. Fat helps to fold the fatty acid molecules, making them occupy more space than the acids saturated fat. Thus, unsaturated fatty acids occupy more space in the cell membrane, increasing the fluidity of the membrane. Polyunsaturated fatty acids in oils are incorporated into cell membranes, altering their physical and functional properties and diminishing the ability of the cells to provide immunity to fight against cancer cells (Sundeep S. Deol *et al.*, 2004).

For therapeutic purposes, the fruits and flowers of the sloe are used, both having astringent, diuretic, depurative, tonic, antiseptic and sedative effects (in cardiovascular diseases). Moreover, fruits (decoction form) and gourd flowers (infusion) contribute to elimination (disintegration) of the uric acid in blood. Considering the health benefits of blackthorn fruits, the purpose of this study was to determine the content of minerals and fatty acids in blackthorn fruits sampled from the vicinity of Cluj-Napoca town, Romania.

MATERIALS AND METHODS

Sampling and sample preparation

The research material consisted of sloe fruits was collected from wild-grown bushes located near Cluj-Napoca, Romania. The fruits were transported in cooled bags to the laboratory.After harvesting, the fruits were washed in tap water and rinsed 3 times with distilled water before the physico-chemical analysis performing.

Analytical methods

The proximate composition (pH, dry matter and ash content) were determined according to AOAC (1995) and Marakoglu *et al*., (2005).

Dry matter. 30 g of sample were dried at 105 °C for 10 h. After drying, the sample was placed in a desiccator for 30 minutes after which it was weighed. Dry matter is calculated by following equation (1):

$$\text{Dry matter (\%)} = \frac{m_2 - m_0}{m_1 - m_0} \times 100 \qquad \text{Eq. 1}$$

where: m_o is the mass of empty capsule(g), m_1 is the mass of capsule containing the dry sample (g) and m_2 is the capsule mass containing dry sample in the oven (g) (Nicholas T. *et al*., 2017).

pH determination. The pH is a significant parameter (Banerjee *et al.*, 2017, Asfaram *et al.*, 2017). 1 g of sample was placed in a 200 mL Erlenmeyer flask. 100 mL water were added and, then, heated under gentle boiling for 15 min. Finally, the solution was cooled to room temperature, diluted with water to 100 mL and the pH was determined immediately with Metller Toledo pH meter (Ekpete *et al.*, 2017). The pH value was 3.64%, Cemerogˇlu *et al.* (1986) reported that the pH is between 2.80–3.95%.

Ash content. The ash content of the fruit samples was determined gravimetrically using the standard calcination furnace for dry ashing protocol (Watson *et al.*, 1994; Latimer J. *et al.*, 2012). 20 g of the oven dried fruit sample were burned at 550°C for 2 h, cooled in a desiccator and weighed. According to equation (2), the ash content was calculated:

$$\text{Ash(\%)} = \frac{m_{ash}}{m_{dry\ sample}} \times 100 \qquad \text{Eq. 2}$$

where:

m_{ash} is the mass of the raw mass of the sample (g) and $m_{dry\ sample}$ is the mass of the dried sample (g) ([(Watson *et al*., 1994; Latimer J. *et al.*, 2012)]).

The crude protein, dry matter and the ash content are affected by the growth conditions (Haciseferogullari *et al.*, 2005).

For the mineral content determinations, a closed vessel microwave system Berghof MWS-3+ (Eningen, Germany) was used. 0.5 g of sample with 6 mL of HNO_3 65% and 3 mL of H_2O_2 30% were added in PTFE vessels, which were kept for 4 h at room temperature before the digestion. Samples were digested using a foursteps digestion program (time [min]/power [W] / T [°C]: 10/700/170; 15/1000/200; 10/1000/160; 10/1000/100; 10 min ventilation). After mineralization, clear solutions were cooled at room temperature, quantitatively transferred to 20 mL volumetric flasks and diluted with deionized water. The Na, K, Ca, Mg and Pcontents were determined by ICP-OES (OPTIMA 5300 DV, Perkin Elmer, USA). The calibration standards were prepared by diluting a stock standard solution (1000 mg L^{-1}) in 0.5% (v/v) HNO_3.

Protein content

The protein content was determined using a Flash EA 2000 CHNS/O analyzer (Thermo Fisher Scientific, USA) by means of combustion of 2 mg of sample. The instrument calibration was performed using atropine (4.84 %N) (Thermo Fisher Scientific, UK) with K factor as calibration method. The protein was calculated using the general factor of 6.25.

Fatty acids

Quantification of fatty acids accumulation was performed by gas chromatography (GC). The fatty acid methyl esters were extracted from 3 g of sample by stirring at the room temperature over 30 minutes with a 2:1 volume ratio of chloroform:methanol. The extraction was derivatized with 3M HCl in methanol for 40 minutes at 80 °C. Analysis of the methyl esters of fatty acids was performed by gas chromatography using a flame ionization detector (GC-FID) according to (Barros *et al.*, 2008). The capillary column used DB-WAX had the following features: 30 m x 0.25 mm x 0.25 mm. The oven temperature for methyl esters was started at 50 °C (5 min) followed by an increase ramp of 10 °C × min -1 to 310 °C and this final temperature was maintained for 7 min. The qualitative and quantitative analyses werebased on a benchmark.

RESULTS AND DISCUSSION

The obtained results of blackthorn samples (*Prunus spinosa*) by gravimetric methods are presented in Table 1. The dry matter content was 18%, while in the flesh, reached 17%, according to the food commodity dictionary from 1957 (Ziemiański *et al.*, 1957).

Table 1

Chemical composition of blackthorn fruits

Properties	Value (%)	Fatty acids	Value (%)	Fatty acids	Value (%)
Dry matter (%)	18.0	C8:0	0.02	C18:0	2.55
Ash (%)	0.58	C10:0	0.03	C18:1n9c	57.4
pH	3.64	C12:0	0.09	C18:2n6c	23.4
Protein (%)	3.2	C14:0	0.08	C18:3n3	2.62
K (mg/kg)	17925	C14:1	0.04	C20:0	0.48
Ca (mg/kg)	1476	C15:0	0.05	C20:1c	0.04
Mg (mg/kg)	896	C16:0	6.48	C20:3n3+C21:0	0.05
P (mg/kg)	1498	C16:1	0.65	C22:0	0.34
Na (mg/kg)	490	C17:0	0.09	C23:0	4.49
Fe (mg/kg)	15.8	C17:1c	0.11	C24:0	0.54

The highest metal content was obtained for K (17925 mg/kg), which is a significant mineral in hypertension risk reduction (Perez *et al.*, 2014). An amount of 1476 mg/kg of Ca was determined. Ca is a very important mineral for the bone system, maintaining a proper bone mass (Shin *et al.*, 2015). Although the main source of Ca are the milk products, fruits are significant sources as well (Hoaghia et al., 2016). The analyzed sample showed low levels of Na (490 mg/kg). Na is an important element in blood pressure regulation, acid-base balance, muscle function, blood volume, nerve impulse transmission and normal cell function. Although, a higher amount of Na as the admitted limit can lead to incorrect functioning of the human body (Elinge *et al*., 2012; Aburto *et al*., 2013).

Studied blackthorn samples were characterized by a Mg amount of 896 mg/kg, which is an important constituent of bones and teeth. Mg helps the parathyroid hormones synthesis and release, and converts the active form of vitamin D, as well as muscle relaxation and tissue respiration. Mg deficiency leads to seizures, tetanus, neuromuscular hyperexcitation or even death (Elinge *et al*., 2012).

Specialty literature shows the fatty acids indicated that polyunsaturated fatty acids reduce total cholesterol but induce lipid oxidation and reduce HDL cholesterol levels when consumed in large quantities (Berto *et al.*, 2015).The obtained results are shown in Table 1. According to the obtained results, the oleic acid (C18:1) was the most abundant acid (57.4%). Furthermore, polyunsaturated fatty acids predominated over monosaturated fatty acids. The blackthorn fruits exhibit high levels of linoleic acid (23.4%)and a lower level of α-linolenic acid (2.62%). Oleic acid can reduce low-density lipoprotein (LDL), helping arteriosclerosis prevention (Bellido *et al.,* 2006). The obtained data are comparable with those reported by Dawson C. *et al.,* 1997; Mason R.*etal*.,1994, (51.3% oleic acid). Despite this, the fatty acids composition can vary substantially between different cultivars (Kaijser *et al*., 2000).

For the protein content, a factor of 6.25 was used to convert the total nitrogen.The protein content of many edible wild fruits is usually lower than 5%, and varies considerably (Cemeroglu *et al*.,1986). The protein content in studied blackthorn fruits was comparable with those founded in stone fruits (peaches – 1%, cherries – 1 g/ 100 g, apricots – 0.9 g/100 g, cherries 0.9 g/ 100 g)

CONCLUSIONS

The studied blackthorn samples were characterized withvariations in the ash content, dry matter, each being rich in one or more minerals and nutrients.Fruits are rich in fatty acids, the oleic acid (C18:1) being predominant. Polyunsaturated fatty acids predominate on monounsaturated fatty acids with high levels of linolenic acid. α-linolenic acid was found in samples in smaller quantities.The study provides evidence of the amount of nutrients and the proper functioning of the human body requires minerals.

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