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Polyphenols extraction from black chokeberry wastes

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Polyphenols are secondary metabolites of plants and are broadly distributed in fruits and vegetables. It is demonstrated that these compounds act as strong antioxidants which determines a variety of their medicinal properties. Wastes of black chokeberry after juice recovery were used to investigate the optimal conditions for maximal yield of polyphenols applying solid-liquid extraction. Parameters such as temperature, medium acidity and type of solvent were varied. Total polyphenol content was assayed according to the Folin-Ciocalteu colorimetric reaction method. The results obtained demonstrated that water and water-ethanol mixtures were suitable for polyphenols extraction from black chokeberry wastes. The increase in temperature, regardless of the solvent, led to increasing polyphenol content in the extracts, reaching 7700 mg/L GAE with 50 % ethanol at ebullition, while acidity had clearly less effect on polyphenols extraction.

Keywords: aronia melanocarpa, solid-liquid extraction, Folin-Ciocalteu method

INTRODUCTION

The importance of the diet in relation to improving human health, healthy aging and promoting quality life has increased the interest of consumers on nutraceuticals rich foods, and especially on fruits and vegetables [1]. Berries are rich sources of a wide variety of phytochemicals. Black chokeberry (*Aronia melanocarpa*) berries are widely studied for their potential as a natural product for food and medicinal use, because of their high contents of phenolic compounds such as procyanidins, anthocyanins, phenolic acids and flavonoids [2]. This phenolic content seems to correlate to the antioxidant activity reported for these berries [3, 4]. In-vitro anti-viral activity of *A. melanocarpa* against type A influenza virus as well as bacteriostatic activity against *Staphylococcus aureus* and *Escherichia coli* have been reported [5]. A very good gastroprotective effect of Aronia fruit juice in rats has been demonstrated, as well as a potential in prevention and control of diabetes mellitus type II and diabetes associated complications [6].

Extracts of *Aronia melanocarpa* have been applied as a natural anti-hypertensive and anti-atherosclerotic drug [7], have shown a pronounced anti-inflammatory effect [8] and also have shown benefits in treatment of cardiovascular disease [9]. The anthocyanin-rich extracts from black chokeberries were reported to inhibit the growth of cancer cells [10].

Berries are often processed into juices, syrups, jams. After juice extraction, many phenolic compounds and especially anthocyanins, are still present in the solid waste [11, 12] and could be valorized by suitable extraction process. Similar valorizations of food by-products by extracting antioxidant polyphenols have been reported for grape mark [13], apple pomace [14] and artichoke wastes [15], for example.

Solvent extraction is a classical technique to recover polyphenols from natural raw sources [16]. Large quantities of solvent and prolonged contact time are usually applied to obtain high extraction degree [15]. Solvents, such as methanol, acetone [17], hexane [18], propanol and ethyl acetate [19] are commonly used for the extraction of phenolics from plants. The properties of the solvent significantly affect the measured total phenolic content and antioxidant capacity [20]. An important parameter is the polarity of the solvent – highest extract yields have been obtained with polar alcohol based solvents [21], but the efficiency of the solvent is strongly dependent on the plant matrix used [13].

The objective of this work was to investigate the influence of different process parameters such as type of solvent (polarity and acidity) and temperature, in order to determine the optimal conditions for the extraction of polyphenols from black chokeberry wastes.

MATERIALS AND METHODS

Reagents

Folin-Ciocalteu reagent (2N), sodium carbonate (>99%) were supplied by Sigma–Aldrich (France);

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ethanol (95%) was provided by Flandre Chimie (France). All other chemicals used (H_3PO_4, KH_2PO_4, K_2PO_4, K_2HPO_4, K_3PO_4) were of analytical grade.

Sample preparation

Frozen *Aronia melanocarpa* berries supplied from the region around the town of Elena (Bulgaria) were used as plant material. For the studies the frozen berries were defrosted at room temperature, coarsely ground and then pressed in a small laboratory extruder to recover the juice. Finally, the waste material was washed out twice with fresh portions of distilled water and let to dry until constant weight at 60 °C in an oven. To obtain samples of the same moisture content for all extraction experiments, the chokeberry wastes were stored hermetically in a dark place at room temperature until further use.

Total phenolic content

The Folin-Ciocalteu colorimetric reaction method was used for the analysis of total phenolics [22]. Each liquid extract was diluted and mixed with Folin-Ciocalteu reagent, and after 5 min sodium carbonate solution was added. The mixture was allowed to stand for 2 h at room temperature before the measurement of the absorbance at 765 nm. Gallic acid was used as standard and a range of gallic acid concentrations from 0 to 500 mg/L was used to prepare the calibration curve (absorbance vs. concentration). Total phenolic content was determined spectrophotometrically on a UVmini 1240 (Shimadzu) and expressed as gallic acid equivalents (GAE) in concentration units (mg/L) and as total polyphenols extraction yield (TP yield, %) from dry aronia waste. The latter was determined from the division of the calculated polyphenols weight to the weight of the waste sample, expressed in percentage.

Experimental procedure

Solid-liquid extraction at different temperatures was studied for a period of 4 h in thermostatic shaker under moderate agitation, which maintained all particles in suspension. At ebullition the extraction of polyphenols was carried out under reflux.

As solvents water and ethanol-water mixtures were used. When necessary, the acidity of the aqueous solvent was adjusted with H_3PO_4 solutions and/or buffer solutions of potassium salts. The experiments were conducted at solid-solvent ratio 1:15 (2 g dry aronia wastes in 30 mL of solvent).

After extraction the two phases were separated by filtration and the liquid extracts were put to analysis by the Folin-Ciocalteu method.

RESULTS AND DISCUSSION

Influence of pH on the extraction of polyphenols

To determine if the acidity of aqueous solutions affects the phenolic extraction from black chokeberry, distilled water with pH 4 (its own acidity), more acidic pH 3 and alkaline pH 10 was tested as solvent. The results are presented in Fig. 1 and Table 1.

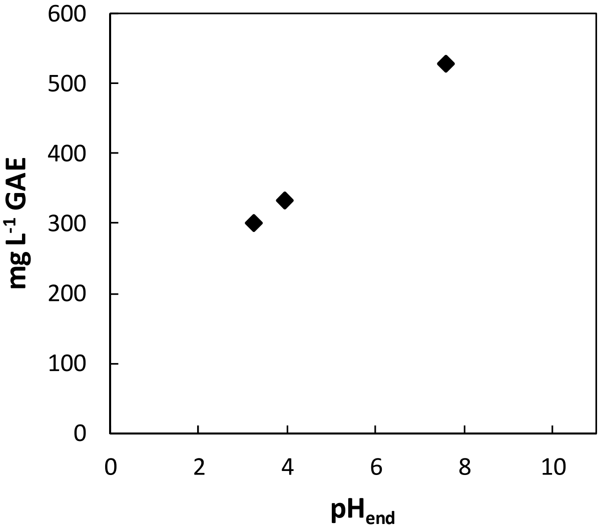

Fig.1. Effect of pH on polyphenols extraction from black chokeberry waste (20 °C, solid-solvent ratio 1:15, 4 h)

The obtained results demonstrate a steady increase (see Fig.1) in the content of the extracted polyphenols with decreasing acidity of the medium and 75 % increase in the extraction yield (see Table 1). The higher results in alkaline solutions could be attributed to the changes in the conformation of some phenolic compounds with pH changes.

Table 1. Total polyphenols extraction yield from dry black chokeberry waste depending on medium pH

Solvent	pH_0, -	pH_{end}, -	TP yield, %
water	3.04	3.25	0.45
water	4.05	3.95	0.50
water	10.00	7.59	0.79

Influence of type of solvent

For these studies distilled water and water-ethanol solutions were chosen as solvents, which are of polar type and are non-toxic as well. It was observed that solvents with ethanol content in the range from 40 to 60 % (vol.) are to be favourably used for extraction of polyphenols from black chokeberry waste (see Fig.2).

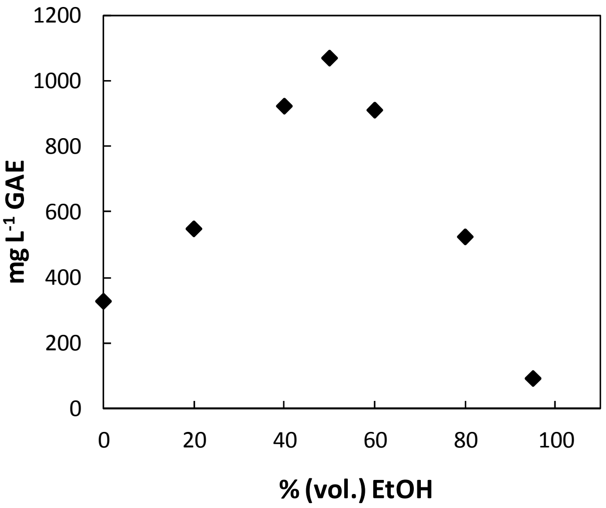

Fig.2. Effect of solvent on polyphenols extraction from black chokeberry waste (20 ºC, solid-solvent ratio 1:15, 4 h)

Best results were obtained with 50 % (vol.) ethanol – the content of polyphenols was 3 times higher than this extracted with pure water and almost 12-fold higher than this with 95 % (vol.) ethanol. In Table 2 the extraction yield of total polyphenols from dry aronia waste is given according to the type of solvent.

Similar tendency in the results with water-ethanol solutions as solvent were observed by Galvan d'Alessandro et al. [4]. They have used directly aronia berries as material and have reported better total polyphenols extraction yield with water than with ethanol, and maximal yield with 50 % (vol.) ethanol.

Table 2. Total polyphenols extraction yield from dry black chokeberry waste depending on the type of solvent

Solvent	TP yield, %
water	0.50
20 % EtOH	0.82
40 % EtOH	1.38
50 % EtOH	1.60
60 % EtOH	1.37
80 % EtOH	0.79
95 % EtOH	0.14

Influence of temperature

The extraction of phenolics from plants is reported to be favourably affected by increasing temperature [4, 23]. To investigate the influence of this parameter, experiments at 20 ºC, 40 ºC and boiling point temperatures were undertaken. Distilled water and 50 % (vol.) ethanol were used as solvents. The obtained results showed not so distinctive difference in the amount of extracted polyphenols at 20 ºC and 40 ºC, especially with water as solvent (see Fig.3). Further increase in temperature had a very pronounced positive effect on phenolics extraction, in particular when 50 % (vol.) ethanol was used - almost 4.5 times higher content of polyphenols was achieved at 73.3 ºC (boiling point) than at 40 ºC. Pure water was also very efficient at boiling point, yet 2 times less effective than 50 % (vol.) ethanol at 73.3 ºC.

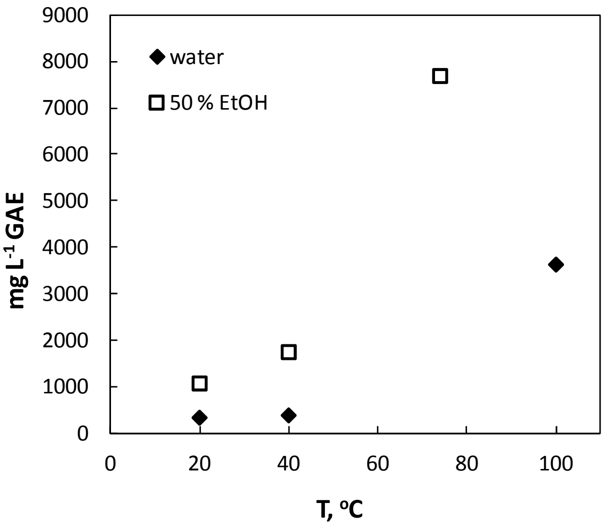

Fig.3. Effect of temperature on polyphenols extraction from black chokeberry waste (solid-solvent ratio 1:15, 4 h)

Higher temperatures enhance solubility of phenolic compounds and their diffusion out of the plant tissue structure, which result in higher total phenol levels [24]. In Table 3 the extraction yield of total polyphenols from dry aronia waste is given according to temperature.

Table 3. Total polyphenols extraction yield from dry black chokeberry waste depending on temperature

Temperature, °C	TP yield, % (water as solvent)	TP yield, % (50 % EtOH as solvent)
20	0.50	1.60
40	0.57	2.62
boiling:		
100 (water)	5.44	
73.3 (50 % EtOH)		11.53

CONCLUSIONS

In this work experimental studies of the solid-solvent extraction of polyphenolic compounds from black chokeberry wastes were carried out. The change of pH of the aqueous solvent affects polyphenols extraction efficiency only in the presence of suitable buffer solutions, and the effect is evidently less than the addition of ethanol to the water. Ethanol containing solutions (20 ÷ 80 %) were found out to be more efficient for polyphenols extraction than pure water or 95 % (vol.) ethanol. The highest content of polyphenols (1070 mg/L GAE) was achieved with 50 % (vol.) ethanol. Temperature showed to be a process parameter to be reckoned with. Under high temperature water can also be efficiently used as well as ethanol containing solutions. At ebullition water and 50 % (vol.) ethanol extracted 3630 mg/L GAE and 7700 mg/L GAE, respectively. These results could contribute to the development of environmentally friendly technologies for polyphenols extraction from fruit wastes and the produced extracts to be used in pharmacy, cosmetics and food industry.

REFERENCES

1 O. Paredes-López, M. L. Cervantes-Ceja, M. Vigna-Pérez, T. Hernández-Pérez, *Plant Foods Hum. Nutr.,* 65, No 3, 299, (2010).

2 D. Rugină, Z. Sconţa, L. Leopold, A. Pintea, A. Bunea, C. Socaciu, *J Med Food*, 15, No 8, 700, (2012).

3 M. P. Kähkönen, A. I. Hopia, H. J. Vuorela, J.-P. Rauha, K. Pihlaja, T. S. Kujala, M. Heinonen, *J. Agric. Food Chem*., 47, 3954, (1999).

4 L. Galvan D'Alessandro, K. Kriaa, I. Nikov, K. Dimitrov, *Sep. Purif. Technol*., 93, 42, (2012).

5 S. V. Valcheva-Kuzmanova, A. Belcheva, *Folia Med.*, 48, 11, (2006).

6 S. Valcheva-Kuzmanova, K. Kuzmanov, S. Tancheva, A. Belcheva, *Methods Find. Exp. Clin. Pharmacol.,* 29, 101, (2007).

7 C. A. Domarew, R. R. Holt, G. Goldmann-Snikoff, *J. Herb. Pharmacother.,* 2, 31, (2002).

8 K. Ohgami, I. Ilieva, K. Shiratori, Y. Koyama, X.-H. Jin, K. Yoshida, S Kase, N. Kitaichi, Y. Suzuki, T. Tanaka, S. Ohno, *Invest. Ophthalmol. Vis. Sci.,* 46, No 1, 275 (2005).

9 D. R. Bell, K. Gochenaur, *J. Appl. Physiol.*, 100, No 4, 1164, (2006).

10 C. Zhao, M. M. Giusti, M. Malik, M. P. Moyer, B. A. Magnuson, *J. Agric. Food Chem.*, 52, No 20, 6122, (2004).

11 J. Oszmianski, A. Wojdylo, *Europ. Food Res. Technol.*, 221, 809, (2005).

12 P. Vauchel, L. Galvan D'Alessandro, P. Dhulster, I. Nikov, K. Dimitrov, *J. Food Eng.*, 158, 1, (2015).

13 G. Spigno, L. Tramelli, D. M. De Faveri, *J. Food Eng.,* 81, No 1, 200, (2007).

14 D. Pingret, A.-S. Fabiano-Tixier, C. L. Bourvellec, C. M. G. C. Renard, F. Chemat, *J. Food Eng.*, 111, 73, (2012).

15 G. Angelov, S. Georgieva, S. Boyadzhieva, L. Boyadzhiev, *C.R. Acad. Bulg. Sci.*, 68, No 10, 1235, (2015).

16 M. Virot, V. Tomao, C. Le Bourvellec, C.M.C.G. Renard, F. Chemat, *Ultrason. Sonochem.*, 17, No 6, 1066, (2010).

17 M. Alothman, R. Bhat, A.A. Karim, *Food Chem.,* 2009, 115, No 3, 785, (2009).

18 B. Pliszka, G. Huszcza-Ciolkowska, E. Januszewicz, I. Warminska-Radyko, *Acta Aliment.*, 42, No 2, 256, (2013).

19 T. Vatai, M. Skerget, Z. Knez, *J. Food Eng.*, 90, No 2, 246, (2009).

20 J. A. Michiels, C. Kevers, J. Pincemail, J. O. Defraigne, J. Dommes, *Food Chem.*, 130, No 4, 986, (2012).

21 L. Tomsone, Z. Kruma, R. Galoburda, *World Acad. Sci. Eng. Technol*., 64 , 903, (2012).

22 V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventos, *Methods Enzymol*., 299, 152, (1999).

23 N. Harbourne, J. C. Jacquier, D. O'Riordan, *Food Chem*., 116, 722, (2009).

24 T. Hofmann, E. Nebehaja, É. Stefanovits-Bányaib, L. Albert, *Ind. Crop Prod.*, 77, 375, (2015).