pH and Thermal Stability of Anthocyanin-based Optimised Extracts of Romanian Red Onion Cultivars

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Abstract

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Total phenolics and anthocyanins in ten samples of Romanian red onion cultivars, extraction optimisation and pH/thermal stability were investigated. Extraction with 80% aqueous ethanol leads to an increased anthocyanin yield. The level of phenolics and anthocyanins in bulbs is highly variable and was found higher in the Red of Turda cultivar. The highest value of 99.66 mg/100 g FM was obtained for red onion dry skins. Strong acidic conditions favoured the better stability of anthocyanin extracts during 10-day storage. The first order rate constant value of anthocyanin degradation during storage calculated at different pH increased almost 17 times when pH increased from 1.0 to 9.0. Results of thermal analysis performed by differential scanning calorimetry showed that temperatures over 45°C already induced anthocyanin degradation in extracts. These results may become useful for establishing the appropriate conditions of processing and storage of anthocyanin-based foods. The obtained data on total phenolics and anthocyanins in *Allium cepa* L. may become relevant for future estimation studies of their daily consumption and for completing the national food composition databases.

Keywords: *Allium cepa* L.; phenolics; anthocyanins; pH differential; Differential Scanning Calorimetry

Onion (*Allium cepa* L.) is one of the oldest and most frequently cultivated food plants highly valued for its pharmacological properties, such as antioxidant, antimicrobial and antitumor ones, reduction of cancer risk and protection against cardiovascular diseases (Lachman *et al*. 1999; Ly *et al*. 2005). Though it is not specifically considered as a medicinal herb, the onion has shown health-promoting effects based on its secondary metabolites, such as flavonoids to which the strong antioxidant properties of onion have been attributed (Lachman *et al*. 2003; Nuutila *et al*. 2003; Kim & Kim 2006). The onion bulb presents the most health-promoting properties when used raw. Besides culinary and medicinal applications, the onion is also used for cosmetic purposes or as herbal dyestuffs in textile dyeing (Vankar & Shanker 2009).

Although onion production and consumption play a key role in the Romanian agri-food system, unfortunately red onions, which have a higher content of phytochemicals of pharmacological interest, have not gained the same popularity as the yellow ones.

Red onions owe their colour mainly to anthocyanins from the epidermal cells of the scale leaves of the bulb. Among flavonoids, anthocyanins are considered the most biologically active compounds, contributing to the high antioxidant potential. Structurally, anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salts, being composed of an aglycon moiety

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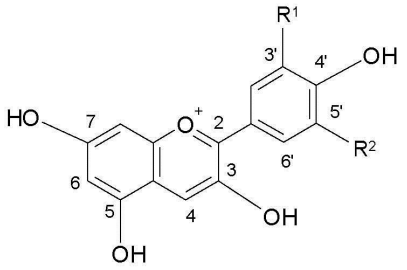

R^1 = H	R^2 = H	pelargonidin
R^1 = OH	R^2 = H	cyanidine
R^1 = OH	R^2 = OH	delphinidin
R^1 = OH	R^2 = OH	peonidin
R^1 = OCH_2	R^2 = OH	petunidin
R^1 = OCH_3	R^2 = OCH_3	malvidin

Figure 1. General chemical structure of anthocyanidin (flavylium cation)

called anthocyanidin (Figure 1) and carbohydrate residues (glucose, rhamnose, xylose, galactose, arabinose, rutinose) (Harbone & Grayer 1988).

Acylated and non-acylated anthocyanins, such as cyanidin mono- and diglucosides, cyanidin 3-laminariobioside, peonidin mono- and diglucosides, petunidin glucoside, and 5-carboxypyranocyanidin 3-glucoside, have been reported to occur in red onions (Slimestad *et al*. 2007). The most frequently reported anthocyanins that occur in different red onions are cyanidin derivatives.

Epidemiological studies regarding antioxidant compounds from food plants have shown their protective effects against chronic diseases. As the level of phytochemicals highly depends on genetic and environmental conditions, epidemiological studies regarding their health benefits must be interpreted through this and also through cultural dietary habits. In this respect, it becomes important to have accurate information on the content of antioxidant compounds such as total phenolics (TP) and total anthocyanins (TA) in foods from particular regions, in order to guarantee success to studies regarding the estimation of the daily consumption of these biomolecules.

Conventional and non-traditional extraction procedures have been described for anthocyanins, finally leading to either an enriched crude pigment extract obtained by solid-liquid partition process particularly used for quantitative analysis by UV-Vis spectroscopy (Giusti & Wrolstad 2001) or to a further purified extract. Extraction techniques may be improved by optimisation of various parameters to obtain high extraction yields. Moreover, as anthocyanins have shown a great potential to be used in nutraceuticals and various foods, stability issues of these molecules become important for practical purposes. Research studies have shown that anthocyanins are unstable being oxidised under the action of various factors such as pH, temperature, enzymes, UV radiation, SO_2, ascorbic acid, or chelating metal ions, resulting in colour change and degradation (Rivas-Gonzalo 2003). Though thermal degradation kinetics of anthocyanins was established as pseudo first-order kinetics (Harborne *et al*. 2008), other researchers (Corrales *et al*. 2008) have shown that the presence of other synergistic antioxidant compounds may provide protective effects of the extracts and foods against anthocyanin degradation.

The aim of the present study was to investigate the TP and TA contents in representative red onion cultivars (edible and non-edible parts) grown in Romania, to establish the optimised extraction conditions and to evaluate the pH and thermal stability of selected pigment extracts and the kinetics of the anthocyanin degradation during storage. pH stability was investigated during storage at room temperature in dark (shelf life of red onion anthocyanin extracts), while the thermal analysis was performed by a differential scanning calorimetry (DSC) technique. These studies may become useful for establishing the appropriate conditions of processing and storage of anthocyanin-containing food products.

MATERIAL AND METHODS

Plant material and reagents. Red onion (*Allium cepa* L.) samples were obtained (2011 and 2012) from traditional markets and local farmers from the following Romanian growing areas: Turda, Făgăraş, Buzău, Drăgăşani, and Sibiu. The regions Turda and Făgăraş situated in Transylvania, Romania, are well known for the local red onion developed cultivars Red of Turda and Red of Făgăraş, respectively. All red onion samples were declared as open air cultivated.

Bulbs and dry outer peels of the investigated red onions were used for analysis.

Chemical reagents of analytical grade without further purification were used for preparing solutions for the analysis of TP and TA. Ethanol (> 96% v/v), methanol (> 99.5% v/v), hydrochloric acid (37%) and sodium acetate (trihydrate) were obtained from AdraChim (Bucharest, Romania), potassium chloride was obtained from Chimopar (Bucharest, Romania),

Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany), anhydrous sodium carbonate was purchased from Scharlau (Barcelona, Spain), while gallic acid was obtained from Fluka (Munich, Germany). Buffer solutions for TA were prepared in distilled water, while solutions for TP analysis were prepared in deionised water.

Physicochemical characterisation of extracts. Moisture content of the investigated samples was determined at 105°C using the ML-50 moisture analyser (A&D Company Ltd., Tokyo, Japan). pH measurements were performed with the pH meter Orion 2-star model (Thermo Scientific, USA).

Extraction and assay of total phenolics. The total phenol content in red onion samples was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton & Rossi 1965). Briefly, the extract in 90% (v/v) methanol was mixed with deionised water and Folin-Ciocalteu reagent and incubated at room temperature for 5 minutes. Then 7% (m/V) Na_2CO_3 solution was added. After incubation at room temperature for 90 min, the absorbance was measured at 745 nm. The T80 UV/VIS spectrophotometer (PG Instruments Ltd., Wibtoft, UK) was used. Gallic acid was used as standard for the calibration curve. A five-point calibration curve of gallic acid in the range of 20–100 mg/l ($y = 0.0046x + 0.0146$ with R^2 of 0.9914) was constructed. The mean of three readings was used and the TP content was expressed in milligrams of gallic acid equivalents per 100 g fresh mass (mg GAE/100 g FM).

Extraction and assay of total anthocyanins. Bulbs and dry outer peels of red onions from each cultivar were grated, and anthocyanins were extracted at 4°C, overnight or for 2 h, depending on the optimised extraction experiment, using different solvent systems: (1) ethanol/acetic acid/water (50/8/42); (2) ethanol/acetic acid/water (70/4/26); (3) ethanol/acetic acid/water (80/1/19); (4) 50% ethanol (v/v); (5) 70% ethanol (v/v), and (6) 80% ethanol (v/v).

The extracts were centrifuged at 8000 rpm at 4°C for 10 minutes. The refrigerated centrifuge (NF 800R model; Nűve, Ankara, Turkey) was used. Extracts were stored at 4°C until analyses. The TA content was determined according to the spectrophotometric pH differential method (Giusti & Wrolstad 2001). Measurements were done in two replicates. Total anthocyanins were expressed as cyanidin 3-*O*-glucoside (Cyn 3-*O*-G) equivalents (mg/100 g FM).

In the pH stability study, buffers of four pH values (1.0, 4.5, 7.4, and 9.0) were prepared. The hydroethanolic extract of anthocyanins was dissolved in buffer solutions in separate 10 ml volumetric flasks and stored in dark at room temperature for 10 days. The absorbance was determined at days 1, 2, 4, 7, 8, and 10.

Differential scanning calorimetric analysis. Differential scanning calorimetry (DSC) was carried out by means of the DSC calorimeter (SDT Q600 model; TA Instruments Inc.; New Castle, USA), calibrated according to the standard procedure using pure indium, under a nitrogen flow stream of 20 ml per minutes. Samples (15–20 mg) were heated in the range of 30–200°C with a heating rate of 10°C/min in sealed platinum pans. Subsequently, each solvent in part, in amounts equivalent to those of the samples was heated similarly. In all experiments, an empty sealed platinum pan was used as reference. The analysed DSC curves were obtained using the curves of the extracting solvents of the investigated anthocyanins samples as baseline. For data collection the TA Instruments Universal analysis software (TA Instruments Inc., New Castle, USA) was employed.

RESULTS AND DISCUSSION

TA and TP content in various red onion samples – Extraction optimisation

As anthocyanins from red onions may have a great potential to be used as nutraceuticals or as eco-friendly dyestuffs, factors influencing their applications in terms of stability are discussed in the present work.

In order to determine the solvent influence on anthocyanins extracted from red onion (*Allium cepa* L.) cv. Red of Turda (Romania), the following solvent systems based on acidified and non-acidified hydroethanolic solution were used: (1) ethanol/acetic acid/water (50/8/42); (2) ethanol/acetic acid/water (70/4/26); (3) ethanol/acetic acid/water (80/1/19); (4) 50% ethanol (v/v); (5) 70% ethanol (v/v), and (6) 80% ethanol (v/v). Extraction was conducted at 4°C. Evaluation of the anthocyanin concentration was performed by the pH differential spectrophotometric method.

As presented in Figure 2, for the selected cultivar, 80% ethanol solution proved more efficient than the other systems regarding the extraction yield of TA. 80% ethanol solution is a safe solvent system which also minimises the pigment decomposition favouring the extraction of TA in their native form.

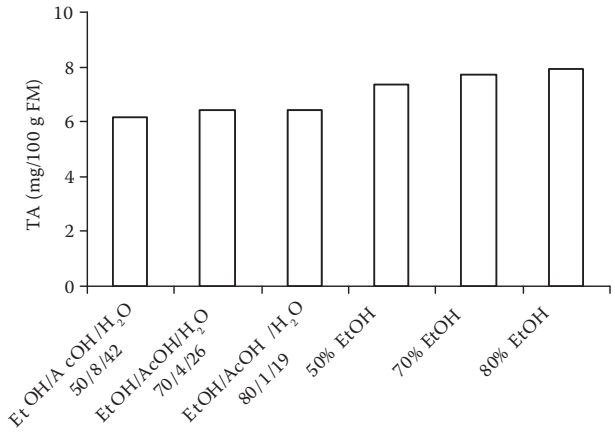

Figure 2. Total anthocyanins content in red onion (*Allium cepa* L. cv. Red of Turda) according to different extraction solvent systems at 4°C

As extraction with 80% ethanol determined an increased anthocyanin content in the investigated red onion bulb sample (*Allium cepa* L. cv. Red of Turda), we further investigated TP and TA contents and physicochemical parameters of samples from other four regional cultivars of red onions using this extracting solvent. Results are shown in Table 1.

As observed in Table 1, red onion samples originating from the same growing area showed small differences in TA and TP contents, except *Allium cepa* L. cv. Red of Turda sample 3, which has the highest concentration of these bioactives (7.93 mg/100 g FM). Samples 3 and 7–9 were purchased in the traditional market in November 2011, while samples 1–2 and 4–6 were purchased in February 2012, which may explain the high amounts of TA found in samples with shorter storage time at local farmers or markets, as these pigments are known to degrade through time. Also, the various concentrations of TA and TP in red onions could be due to the applied agricultural practices (grown from seed or transplanted, use of fertilisers), production (local farmers, small vegetable gardens), harvest and postharvest practices, and probably genetic factors. Accumulation of anthocyanins in red onions may change also with environmental conditions (temperature, rainfall patterns, soil water content) (Patil *et al*. 1995).

Literature reported the values of TA content in red onions varying from 0.26–0.59 mg TA/100 g FM (HPLC method) (Rodrigues *et al*. 2011) to 25 mg/100 g FM (Timberlake & Henry 1988) and even higher amounts of 48.5 mg/100 g FM found in some varieties (Wu *et al*. 2006). Regarding the TP content in red onions, literature data reports values between 154.1 and 310.8 mg GAE/100 g FM (Marinova *et al*. 2005; Lin & Tang 2007). Differences between variously coloured Czech cultivars have been reported; average TP content in cv. Ala (white) was the lowest, followed by cv. Všetana (yellow) and the highest was in cv. Karmen (red) – 2645 mg/100 g DM, 6521 mg/100 g DM and 10 830 mg/100 g DM, respectively (Lachman *et al*. 2003).

Considering that the future textile dying application of red onion anthocyanin extracts may be intended, we also evaluated the TP and TA contents

Table 1. Physicochemical characterisation, TA and TP contents in fleshy bulbs (1–9), and dry outer peels (10) of different Romanian red onion (*Allium cepa* L.) cultivars

Sample No.	Romanian region of cultivation	Moisture (g/100 g)	pH of the extract	TA (mg/100 g FM)	TP (mg GAE/100 g FM)
1	Turda	86.5	6.16	2.30	174.2
2	Turda	89.1	5.84	0.12	141.5
3	Turda	90.2	6.15	7.93	197.5
4	Făgăraș	90.4	5.99	2.36	159.2
5	Făgăraș	90.0	6.08	2.36	158.2
6	Făgăraș	87.0	6.18	1.35	nd
7	Făgăraș	89.5	6.05	1.01	nd
8	Buzău	90.1	6.00	2.05	147.5
9	Drăgășani	90.0	6.15	6.19	185.7
10	Sibiu	14.9	3.80	99.66	1345.74

FM – fresh matter; GAE – gallic acid equivalents; nd = not determined

in dry outer peels removed from a sample (10) obtained from the Sibiu region of Romania. The determined TP content was 1345.74 mg GAE/100 g FM, while the TA content in the anthocyanin extract in ethanol/acetic acid/water 50/8/42 (2 h at 4°C, solvent/sample ratio of 4) was found the highest (99.66 mg/100 g FM).

Effect of pH on the stability of red onion anthocyanin extracts

Regarding the effect of pH on the stability of anthocyanin-based crude extracts from red onions, we selected the sample of *Allium cepa* L. cv. Red of Turda and investigated using four different buffer solutions of pH 1.0, 4.5, 7.4, and 9.0, respectively. In order to eliminate the concentration step of the anthocyanin extract which may lead to degradation, for this experiment we used low solvent volumes of 80% ethanol (v/v) for extraction, so the lower TA content was explained.

The TA content of each buffer solution of anthocyanins determined during 10-day storage in the dark at room temperature is presented in Figure 3. Precipitates (colourless sediments) formed during storage, which may favour enzymatic or non-enzymatic (browning) degradation, were removed by centrifugation before each analysis. After an apparent initial increase in TA content of the crude extract under strong acidic conditions (pH 1.0), TA content decreased gradually in the investigated samples during the remainder of the storage period in the dark, with significantly lower final TA level in the extract in the pH 9.0 buffer solution. This is explained by an increase of the red flavylium cation concentration in acidic media and its possible interaction with existing copigments, which affects the absorption properties of the anthocyanin solutions and ensures an improved extract stability and colour protection through time compared to the other investigated solutions. The results demonstrated that anthocyanins in red onion extract were more stable at low pH values (1.0) and highly unstable under alkaline conditions (pH 9.0). As previously reported, under strong acidic conditions, anthocyanins exist as flavylium cation which showed lower degradation (Hubbermann *et al*. 2006). These results are in agreement with those of most studies regarding the stability of anthocyanins extracted from different plants (Delgado-Vargas & Paredes-López 2003).

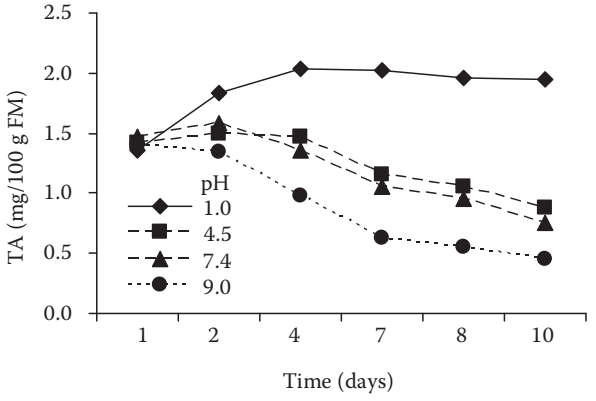

Figure 3. Effect of pH on the stability of stored anthocyanin hydroethanolic extract from red onion sample of Turda, Romanian region

Regarding the colour stability of anthocyanin extracts in different buffer solutions during storage, the extracts at pH 1.0 and 4.5 still showed an attractive red-violet colour after 10 days of storage at room temperature in the dark. The sample at pH 9.0 showed a significant increase of the absorbance measured at 420 nm, which represents the chalcone and browning region.

Colour stability of anthocyanins at a given pH mainly depends on the structures of anthocyanins. At pH 1.0, anthocyanins are in the structural form of flavylium cation, while with increasing pH (7.4) the structural form of the coloured quinonoidal form is favoured by the rapid proton loss. Through time (pH 4.5), the flavylium cation yields the hydrated colourless carbinol form, which becomes equilibrated to the open colourless chalcone form (Mazza & Miniati 1993).

Kinetics of degradation of anthocyanins in red onion anthocyanin extracts during storage

Degradation of anthocyanins during storage is reported to follow the first order kinetics (Daravingas & Cain 1968; El-Kady & Ammar 1977; Wang & Xu 2007; Jiménez *et al*. 2010). The reaction rate (r) expression for the first order reaction was determined to be proportional to the concentration of a single reactant (A) raised to the first power (Eq. 1).

$$r = \frac{d[A]}{dt} = k[A] \quad (1)$$

where: k – 1st order rate constant, which has units of s^{-1}

Table 2. The calculated rate constants as a function of pH of the anthocyanin hydroethanolic extract from red onion samples of *Allium cepa* L. cv. Red of Turda.

pH	$k \times 10^{-3}$ (day^{-1})	Dry residue by thermogravimetry (at 105 °C)
1.0	7.74	nd
4.5	61.3	3.6730
7.4	89.0	1.4010
9.0	129.0	0.6313

nd – not determined

Table 3. Correlation coefficients at different pH of the anthocyanin hydroethanolic extract obtained using values of constant values to recalculate TA content for different time intervals

pH	Correlation coefficients
1.0	0.83673
4.5	0.99996
7.4	0.99689
9.0	0.99754

By arranging the terms with separation of the variables in Eq. 1, Eq. 2 is obtained:

$$\frac{d[A]}{dt} = -kdt \quad (2)$$

Integration from the A_0 concentration and time t_0 to the concentration A at time t leads to Eq. 3 and Eq. 4:

$$\int_{[A_0]}^{[A]} \frac{d[A]}{[A]} = k \int_{t_0}^{t} dt \quad (3)$$

$$\ln[A] - \ln[A_0] = -k(t - t_0) \quad (4)$$

After rearrangement of Eq. 4 and considering $t_0 = 0$, the result is Eq. 5:

$$\ln \frac{d[A]}{[A_0]} = kt \quad (1)$$

The first order rate constants calculated using Eq. 5 for the anthocyanin hydroethanolic extracts from red onion samples of *Allium cepa* L. cv. Red of Turda in different pH buffer solutions are presented in Table 2. Initial concentrations (A_0) were considered the highest values of TA content, then these values started to decline. The values of first order rate constants (k) were calculated as arithmetic means with standard deviations of less than 6.5×10^{-3}. The determined k value increased almost 17 times with the increase of pH from 1.0 to 9.0.

Very high correlation coefficients were obtained while recalculating the concentration of TA for different time intervals using the rate constant values, as shown in Table 3.

DSC investigation of red onion anthocyanin extracts at different pH

As most of the previously reported studies regarding the temperature effect on the stability of anthocyanins were done by placing diluted samples at different temperatures and recording the change in absorbance, we further investigated thermal stability using a different method, the

Table 4. Onset temperatures, peak temperatures and integrated peak values for the 2nd order derivatives of DSC thermograms of the anthocyanin extract of red onion bulb (sample 3) at different pH

pH 4.5			pH 9.0		
Onset temperature (°C)	peak temperature (°C)	integrated peak (mW min/g/ °C^2)	onset temperature (°C)	peak temperature (°C)	integrated peak (mW min/g/°C^2)
–	–	–	44.64	46.69	0.0001126
51.74	55.76	0.01441	51.59	55.46	0.01422
61.97	66.76	0.01330	61.74	66.41	0.01285
73.67	78.43	0.01340	73.76	78.01	0.008736
–	–	–	84.90	89.81	0.004756
93.33	99.11	0.03884	94.09	98.82	0.02839
118.35	133.23	0.01325	119.32	133.29	0.007416

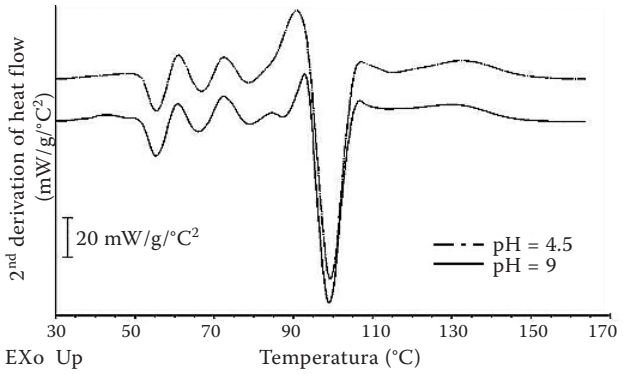

Figure 4. DSC thermograms (2nd order derivatives) of anthocyanin extract of red onion bulb (sample 3) at different pH values

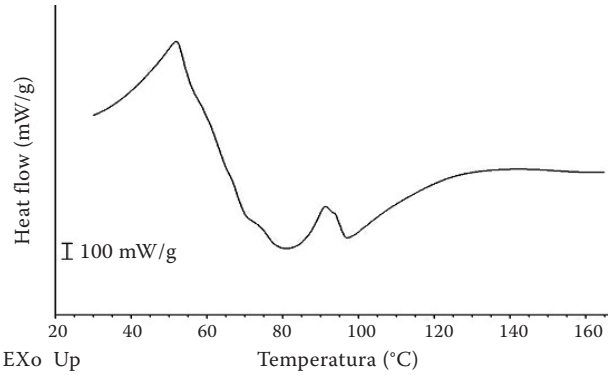

Figure 5. DSC thermogram of anthocyanin extract of red onion dry outer peels (sample 10)

DSC technique applied directly to anthocyanin extracts. As inflections of the DSC curve in thermal analysis experiments indicate a thermal event, the second order derivative of the DSC curve was used, so all these events can be more clearly observed (Gabbott 2007).

The influence of pH on anthocyanins extracted from *Allium cepa* L. cv. Red of Turda (sample 3) using 80% ethanol was first envisaged for this thermal analysis study, as the stability of anthocyanins was shown to be highly influenced by pH. For this reason, buffer solutions were added to sample 3 in order to obtain two different pH values: 4.5 and 9.0, respectively. In each case, the initially obtained DSC curves for ethanol solutions containing equivalent amounts of buffer solutions were subsequently subtracted from the obtained DSC curves for sample 3 at the selected pH. The resultant thermograms are shown in Figure 4, while the numerical values of the onset temperatures, peak temperatures and integrated peaks are indicated in Table 4.

The data presented in Table 4 showed that onset temperatures and peak temperatures are generally similar at both pH values, with slightly lower values at pH 9.0. In the case of anthocyanin extract at pH 9.0, an additional signal was observed. This may indicate a slightly lower stability of anthocyanins under basic conditions. The results showed that degradation of anthocyanin extracts starts at 44.64°C at pH 9.0 compared to 51.74°C, which is the first onset temperature obtained for the extract at pH 4.5. As the obtained results may be influenced by the small amounts of anthocyanins finally found in the analysed sample, we also performed thermal investigation on sample 10, which represents the anthocyanin extract of the dry outer peel of red onions showing the highest TA content (99.66 mg/100 g FM). The anthocyanin extract derived from sample 10 and subjected to thermal analysis was prepared using EtOH/AcOH/H_2O (50/8/42) as extraction solvent. The resultant DSC thermogram after baseline subtraction corresponding to the applied solvent system is shown in Figure 5.

The recorded numerical values of onset temperatures, peak temperatures and integrated peak are presented in Table 5. As shown in Figure 5, above 100°C glass transition with the onset temperature of 119.58°C and glass transition temperature of 127.05°C was observed.

Temperature represents a key factor in the extraction of anthocyanins under different conditions (solvents, pH) as it increases the mass transfer and thus diminishes the extraction time. However, increased temperatures might lead to degradation of anthocyanins. As resulted from our investigations on red onion anthocyanin extracts, an increase of temperature above 45–50°C depending on pH of the obtained extract may produce anthocyanin decomposition. Although there is a scarcity of DSC direct investigations of anthocyanin extracts, other studies regarding the optimisation of anthocyanin extraction showed that although an increased extraction temperature of anthocyanins improves the process efficiency, at a critical temperature of 35°C anthocyanin degradation initiates (Cacace & Mazza 2003), while some researchers showed that temperatures above 70°C cause rapid degradation of these pigments (Havlikova & Mikova 1985). For this reason, recommended conventional extraction of anthocyanins should be done at tem-

Table 5. Numerical values of onset temperatures, peak temperatures and integrated peak of DSC thermograms of the anthocyanin extract in EtOH/AcOH/H_2O (50/8/42) of red onion dry outer peels (sample 10)

Onset temperature (°C)	Peak temperature (°C)	Integrated peak (J/g)
45.52	52.25	10.9800
71.79	74.61	0.2459
86.63	91.25	5.695

peratures from 20°C to 50°C, as agreed by most authors (Dai & Mumper 2010).

CONCLUSIONS

The results regarding the optimisation of conventional anthocyanin extraction from red onion (*Allium cepa* L. cv. Red of Turda, Romania) showed that extraction at 4°C in 80% aqueous ethanol proved more efficient than the other systems regarding the extraction yield of TA. Ethanol solution (80%) is a safe solvent system which also minimises the pigment decomposition favouring the extraction of TA in their native form. Various concentrations of TA and TP were found for the selected red onion bulbs from different Romanian regions, probably due to the applied agricultural, production, harvest and postharvest practices, and genetic factors. Results have shown an increased TA and TP content in the cv. Red of Turda. However, the highest TA content was found for the anthocyanin extract from the dry outer peel parts of red onion (99.66 mg/100 g FM).

The pH stability study performed during 10-day storage of anthocyanin extracts at four different pH showed a significant decrease of TA content at pH > 4.5. Degradation of anthocyanins during storage is reported to follow the first order kinetics. The determined *k* value increased almost 17 times with the increase of pH from 1.0 to 9.0. Very high correlation coefficients were obtained while recalculating the concentration of TA for different time intervals using the constant values.

Thermal degradation of red onion anthocyanins investigated by DSC was found to start at 44.64°C in the case of 80% ethanol extract at pH 9.0 compared to 51.74°C for the same extract at pH 4.5.

The obtained data on total phenolics and total anthocyanins in *Allium cepa* L. may become relevant for future estimation studies of phenolics/anthocyanins daily consumption and for completing the national food composition databases. Although red onion bulbs are good sources of anthocyanins, concentrations of these bioactives are highly variable. The dry skin of red onions is non-edible but accumulates high levels of anthocyanins, showing a great potential for developing cheap natural bioactive ingredients with functional properties. The pH and thermal stability studies may become useful for establishing the appropriate conditions of processing and storage of anthocyanin-containing food products.

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