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INVESTIGATIONS ON THERMAL DEGRADATION OF PRUNUS SPINOSA PHYTOCHEMICALS BY FLUORESCENCE SPECTROSCOPY AND INACTIVATION KINETICS

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The present study focuses on the heat-induced structural changes and the degradation kinetics of polyphenolic compounds correlated antioxidant activity from *Prunus spinosa* fruit extract at temperatures ranging from 60 to 90°C. The influence of heating on anthocyanins was evaluated by using fluorescence spectroscopy technique. The most effective fluorescence intensity was observed at the UV absorption maxima of 300 nm, whereas the extract exhibited a fluorescence emission maximum at 408 nm. By increasing the temperature, a significant decrease in the fluorescence intensity was registered, accompanied by a 12 nm blue-shift at 60°C, followed by 3-8 nm red-shift at 70°C and 80°C and again a 2 nm blue-shift at 90°C, when excited at 270 nm. The presence of the chalcone derivative induced by thermal treatment was also suggested. First-order and fractional conversion kinetic models were used to describe the heat-induced changes of the phytochemicals and antioxidant activity in terms of reaction rate constant and activation energy. A higher degradation rate for polyphenols was observed, whereas the antioxidant activity degrades at the lowest rate. The activation energies for anthocyanins, polyphenols, flavonoids and antioxidant activity were 6.95±1.22, 5.99±1.20, 42.50±9.42 and 13.35±5.01 kJ/mol, respectively. A higher E_a value demonstrated a higher stability of flavonoids at lower temperatures, while a faster degradation rate was observed at higher temperatures.

Keywords: Blackthorn, polyphenols, anthocyanins, antioxidant activity, fluorescence, degradation kinetics

Introduction

Prunus spinosa L. commonly known as sloe, blackthorn, prunellier and endrino is a fruit of the *Prunus* genus, *Rosaceae* family. Its fruits are rich in bioactive compounds showing a great number of beneficial effects, due to their antioxidant properties. Blackthorn fruits are particularly rich in antioxidants, such as vitamins C and E, carotenoids, anthocyanins and flavonoids. These compounds present a series of beneficial actions for the human body, for example can reduce the bad cholesterol (Xia et al., 2007), prevent macular degeneration and cataracts (Fursov

et al., 2005), reduce the risk for certain cancers (Kamei et al., 2009; Shih et al., 2005), and may decrease the incidence of type two diabetes (Anderson and Markham, 2006). *P. spinosa* fruits can be used in traditional medicine because of their astringent, diuretic and purgative properties (Kumarasamy et al., 2004). Pinacho et al. (2015) reported that *Prunus spinosa L.* contains substantial quantities of phenolic antioxidants, including specifically, flavonol heterosides (quercetin and kaempferol), phenolic acids (neochlorogenic and caffeic derivatives), coumarin derivatives as aesculetin, umbelliferone and scopoletin, anthocyanins and type A proanthocyanidins which are a class of secondary metabolites consisting of units of flavan-3-ol bound together by one or two interflavan bonds. Polyphenolic compounds have numerous effects on the treatment and prevention of several diseases like: cardiovascular (Kuriyama et al., 2006; Mursu et al., 2008), anti-ulcer prevention (Zakaria et al., 2011), anti-thrombotics (Han et al., 2012; Tao et al., 2012), anti-inflammatory (Beara et al., 2012; Zimmer et al., 2012), antiallergenic (Chung and Champagne, 2009; Schmitz-Eiberger and Blanke, 2012), anticoagulants (Bijak et al., 2011), immunomodulators (Schütz et al., 2010), antimicrobial (Silva et al., 2012; Xia et al., 2011), vasodilators and analgesic activities (Santoz et al., 2010). The most important beneficial effect is considered to be the anticarcinogenic effect (Jeong et al., 2011; Chen et al., 2011).

In the last years, the interest for functional products has increased, and this is why the food industry is trying to produce foods with numerous health benefits. Regarding the chemical diversity of antioxidant compounds present in foods and the interactions between molecules, the evaluation of total antioxidant capacity represents a useful marker for obtaining new functional products (Egea et al., 2010). These research studies are of great importance for the food industry in the perspective of developing new functional ingredients or new foods. However, despite these advantages, polyphenols are very sensitive to heat and light and present a high metabolism rate and rapid elimination from the body. The main factors affecting the polyphenols stability are: the chemical structure, concentration, temperature, pH, light, oxygen, the nature of the solvent, the presence of enzymes, proteins and metal ions (Rein, 2005).

Thermal treatment is a frequent process used in the food industry, applied to ensure the safety and preservation criteria. However, it is well known that heat treatment may lead to significant losses of the biologically active compounds and therefore of the antioxidant activity. Considering the potential use of blackthorn in the food industry to develop new functional ingredients or products, the aim of the present study is to investigate the thermal degradation kinetics of the total phenolic content (TPC), total anthocyanin content (TAC), total flavonoid content (TFC), and antioxidant activity (DPPH-RSA) of blackthorn extract in the temperature range between 60°C and 90°C for a specific heating time (0-25 minutes). Fluorescence spectroscopy was used as an alternative technique in order to deepen the knowledge of the heat-induces changes in the anthocyanin structure from *Prunus spinosa* extract.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, sodium carbonate, sodium hydroxide, sodium acetate, sodium nitrite, potassium chloride, aluminum chloride, gallic acid, potassium persulfate, formic acid, ethanol and methanol (HPLC grade) were obtained from Sigma Aldrich Steinheim, Germany.

Prunus spinosa fruits

Prunus spinosa fruits were purchased from the local market (Galati, Romania) between October – November 2014. The fruits were washed and then freeze-dried using a Martin Christ Alpha 1-4 freeze dryer.

Phytochemicals extraction

The extraction of phytochemicals from freeze-dried blackthorn was performed according to a previously described procedure (Turturică et al. 2016). In brief, 1 g of freeze-dried blackthorn was homogenized with 8 mL of ethanol (70%) and placed on an orbital shaker at room temperature for 4 hours. The extract was then filtered through 0.45 µm membranes.

Heat treatment

Heat treatment was performed in Eppendorf tubes using a thermostatic water bath (Raypa Trade BBO-4, Barcelona, Spain). In particular, for the fluorescence spectroscopy experiments, 100 µL of extract solutions were heated at temperatures ranging from 60 to 90°C for 15 min, whereas for the thermal degradation kinetics studies, 200 µL extract was heated in the same temperature range for different treatment times (0-25 min). After the thermal treatment, the samples were immediately placed in a mixture of ice and water, to prevent further degradation of bioactive compounds.

Phytochemicals and antioxidant activity analysis

The TPC, TAC, TFC and DPPH RSA of the blackthorn extracts were determined as previously described by Turturică et al. (2016).

High-performance liquid chromatography (HPLC) analysis of anthocyanins

Chromatographic analysis was performed using a Thermo Finnigan Surveyor HPLC system, equipped with an Xcalibur software system (Thermo Scientific, SUA). The anthocyanin extracts were analysed at a wavelength of 520 nm. The used column was a C 18 BDS Hypersil (150 mm x 4.6 mm, 5 µm). The elution step was made with 10% formic acid (A) and 100% methanol (B). The injection volume was 10 µL, and the flow rate was 900 µL/min.

Spectroscopic measurements

Fluorescence measurements were carried out using a LS-55 luminescence spectrometer (PerkinElmer Life Sciences, Shelton, CT, USA) in a 10 x 10 mm quartz cuvette, by diluting 100 µL of sample solutions to 3 mL of 10 mM phosphate buffer (pH 4.0). The fluorescence spectra were measured at the excitation wavelengths of 270 nm and 300 nm. The scan speed was 1000 nm·min^{-1} and the slits had 10 nm.

Mathematical models and kinetic analysis

The degradation kinetics of TFC and DPPH-RSA were described by fitting the experimental data to the first order kinetic model (Eq. 1):

$$\frac{C}{C_0} = e^{-kt} \quad (1)$$

where, C is the parameter to be estimated, the subscript *0* indicates the initial value of the parameter, t is the heating time, and k is the rate constant at T temperature (1/min).

The degradation kinetics of TPC and TAC were described by fitting to a first-order fractional conversion kinetic model. In this model, the changes of blackthorn TPC and TAC (C) as a function of the heating time were described by Eq. 2:

$$C_t = C_\infty + (C_i - C_\infty)exp(-kt) \quad (2)$$

where C_∞ is the equilibrium value at an infinite heating time (the value after which even by prolonging the heating time does not result in any changes of the C value) and C_i represents the TPC and TAC values of the samples at time 0.

The half-life ($t_{1/2}$) of the reaction was calculated assuming the first-order kinetics according to Eq. 3:

$$t_{1/2} = -\ln 0.5 / k \quad (3)$$

The Arrhenius model was used to describe the temperature dependence of both degradation rate constants as described by Eq. 4:

$$k = k_{ref} \exp\left[-\frac{E_a}{R}\left(\frac{1}{T}\right)\right] \quad (4)$$

Where: E_a is the activation energy (kJ/mol), k_{ref} is the reaction constant at the infinite temperature (1/min), T is the absolute temperature (K), and R the universal gas constant (8.314 J/mol·K). The kinetic parameter E_a was estimated by a linear regression of the natural logarithm of the degradation rate constant versus the reciprocal of the absolute temperature. The validity of these equations was evaluated by visual inspection of the linearity of the graph, regression coefficients and residual analysis (Turturică et al., 2016).

Statistical analysis of data

All experiments were performed in triplicates. The results were expressed in terms of average values. Statistical analysis of data was performed using the data analysis tool pack of the Microsoft Excel software.

Results and discussion

Qualitative analysis based on chromatography of Prunus spinosa anthocyanins

Anthocyanins qualitative analysis was accomplished by comparing the retention times and the UV-Vis spectra of the samples to the standards. The quantitative analysis was performed by using standard calibration curves obtained from cyanidin-3-glucoside and cyanidin-3-rutinoside. Figure 1 shows the chromatogram

of blackthorn extract obtained at 520 nm. The chromatogram revealed a number of 5 peaks which correspond to the following anthocyanin compounds: cyanidin-3-glucoside (peak 1, 53.93 µg/g), cyanidin-3-rutinoside (peak 2, 140.35 µg/g), peonidin-3-glucoside (peak 3, 0.23 µg/g), peonidin-3-rutinoside (peak 4, 5.47 µg/g) and an unidentified compound.

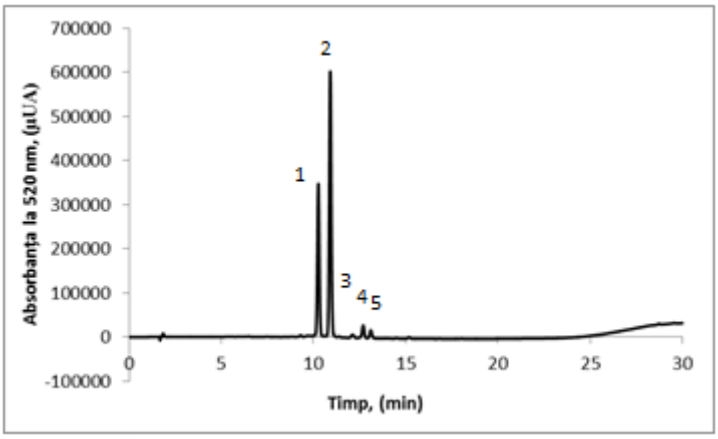


Figure 1. HPLC separation of anthocyanins from blackthorn extract monitored at 520 nm. Peak identification: (1) cyanidin-3-glucoside; (2) cyaniding-3-rutinoside; (3) peonidin-3-glucoside and (4) peonidin-3-rutinoside; (5) unidentified compound

Ruiz-Rodríguez et al. (2014) and Määttä-Riihinen et al. (2004) identified the major anthocyanins in P. spinosa extract as being cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside and peonidin-3-rutinoside. Fraternale et al. (2009) suggested that the most representative anthocyanins in P. spinosa fruit juice were cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside.

Phytochemicals content of the extract

The blackthorn extract had a TPC of 100.6±1.6 mg/100 g dw, which is lower than those reported by the literature. For example, TPC of the extracts was in the range of 21.30 ± 0.60 mg/g to 732.34 ± 6.41 mg/g, as reported by Pinacho et al. (2015). In the three analysed organs (branches, leaves and fruits), the TPC was higher when the extraction solvent was ethanol, followed by water. The TPC in ethanol (732.34 ± 6.41 mg/g) and aqueous extract from branches (499.23 ± 1.99 mg/g) were significantly higher than fruits (359.11 ± 2.54 mg/g and 327.02 ± 4.66 mg/g) and leaves (228.56 ± 2.22 mg/g and 101.28 ± 1.94 mg/g).

The TAC of the blackthorn extract was 14.7±0.003 mg CGE/100 g dw. Määttä-Riihinen et al. (2004) reported an anthocyanin content of 54 mg/100 g fresh sample, whereas Ruiz-Rodriguez et al. (2014) suggested a variable content between 1128.6 - 2585.3 mg CGE/100 g dw. Pinacho et al. (2015) found the highest TAC for the fruits extracted in water (179.00±3.61 mg/g), followed by the fruits extracted in ethanol (165.73±4.21 mg/g) and finally lower values for the leaves

extracted in water (41.50±0.40 mg/g) and leaves extracted in ethanol (40.81±1.26 mg/g).

A TFC of 67.5±0.12 mg CE/100 g dw was found in our study, whereas Pinacho et al. (2015) reported values that ranged from 35.51±1.18 to 554.82±4.51 mg/g.

The antioxidant activity of the blackthorn extracts was determined by DPPH method. The extract exhibited strong scavenging activity against DPPH radicals, having a value of 60%, which corresponds to 23.47±0.145 μmol Trolox/mL. Veličković et al. (2014) studied the influence of solvent on the extraction of phytochemicals from Prunus spinosa L. from the southeastern region of Serbia and reported values ranging from 32.05±0.85% for water extract to 89.10±0.02% for methanol extract. These authors suggested a lower value, of 47.38±0.02% for the extraction in ethanol.

The variation between results in comparison to the data from the literature is caused by the difference of the growing conditions such as soil, geographical position, and environmental conditions during the fruit development, degree of maturity at harvest.

Fluorescence spectra

The fluorescence spectra of polyphenols present in the blackthorn extract were measured at excitation wavelengths of 270 nm and 300 nm as shown in Figure 2.

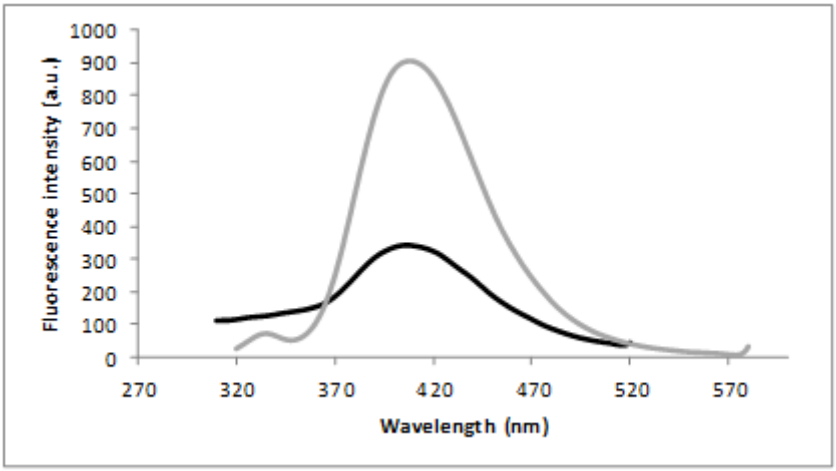
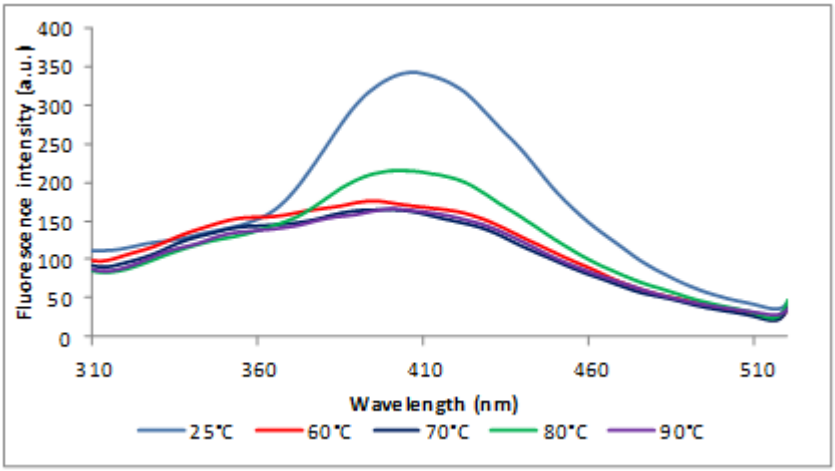
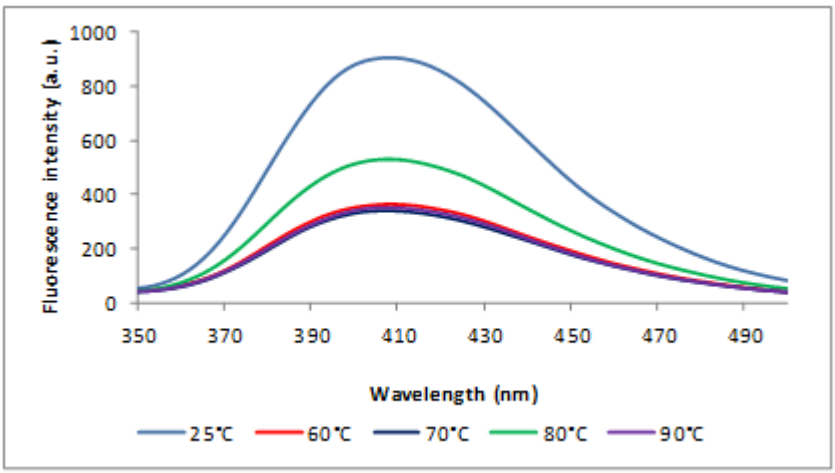


Figure 2. Fluorescence spectra of the blackthorn extract solutions in phosphate buffer at pH 4.0. The excitation wavelengths were 270 nm (black) and 300 nm (grey)

As it can be seen, the most effective fluorescence intensity of the extract was at the UV absorption maxima of 300 nm. Our results suggested that blackthorn extract has a fluorescence emission with one band, at λ_{max} of 407 nm when excited at 270 nm and at 408 nm when excited at 300 nm (Figure 2). In Figure 3 are shown the fluorescence spectra of the heat-treated extracts that resulted from the excitation at 270 nm (a) and 300 nm (b). Heat treatment caused a significant decrease of the blackthorn extract fluorescence intensity when excited at 270 nm (Figure 3 a).



(a)



(b)

Figure 3. Fluorescence spectra of the heat treated blackthorn extract solutions at different temperatures after 90 min of heating. The excitation wavelengths were 270 nm (a) and 300 nm (b)

The thermal treatment can change the structure of anthocyanins to a colorless chalcone derivative, since the reactions are heat sensitive (Timberlake and Bridle, 1980). Regarding the change in wavelength at which the maximum fluorescence is found, a significant 12 nm blue-shift was recorded when heating at 60°C, followed by a 3-8 nm red-shift at temperatures between 70°C and 80°C and again a 2 nm blue-shift at 90°C. Figure 3 (b) shows the emission spectra of the heat-treated samples, followed by the excitation at 300 nm. The increased temperature led to a decrease of the fluorescence intensity, the lowest value being recorded at 90°C. No significant shift occurred in the λ_{max}. Costa et al. (2015) reported that the excitation in the 300-340 nm range (chalcone absorption) induced two emission bands at 420 nm and 495 nm, which are assigned to the isomeric chalcone and to the ionized chalcone forms of the anthocyanins, respectively. Turturică et al. (2016) also

reported significant variations of the fluorescence intensity induced by the heat treatment on anthocyanins from plums. The degradation of anthocyanins above 60°C was explained by Simponson (1985) and involved the hydrolysis of the 3-glycosidic linkage that produces amore labile aglycone and the hydrolytic opening of the pyrylium ring to form a substituted chalcone, which degraded to a brown insoluble compound of a polyphenolic nature.

Kinetic analysis of phytochemical content

The kinetic parameters describing the heat-induced changes of phytochemicals were the degradation rate (1/min) and degradation energy of activation (E_a). Degradation kinetics of the TPC and TAC of the blackthorn extract were modeled using the fractional conversion kinetic model (Eq. 2) (Figure 4 a and b), whereas the first-order kinetic model (Eq. 1) was used to characterize the thermal degradation of TFC and DPPH-RSA (Figure 4 c and d).

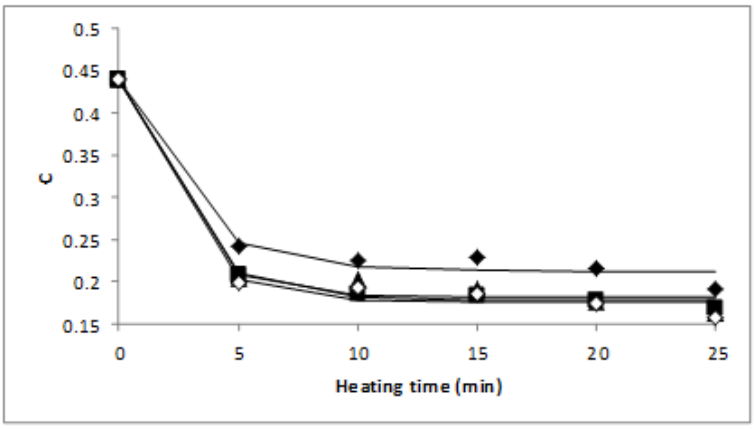
For the TPC, an increase from 38.24±10.83·10^{-2} 1/min at 60°C to 45.63±12.64·10^{-2} 1/min at 90°C was recorded suggesting a higher thermal sensitivity of polyphenols with the increasing temperature (Table 1). Anthocyanins degraded at a lower rate when compared to polyphenols, with *k* values ranging from 31.97±8.90·10^{-2} 1/min at 60°C to 39.14±8.91·10^{-2} 1/min at 90°C. In the case of TFC and DPPH-RSA, the kinetic rate constants increased with the increasing temperature, ranging from 2.62±0.24·10^{-2} 1/min and 1.15±0.14·10^{-2} 1/min at 60°C to 9.02±1.02·10^{-2} 1/min and 2.53±0.28·10^{-2} 1/min, respectively at 90°C.

Table 1. Estimated kinetic parameters (*k*) and activation energy of blackthorn phytochemicals

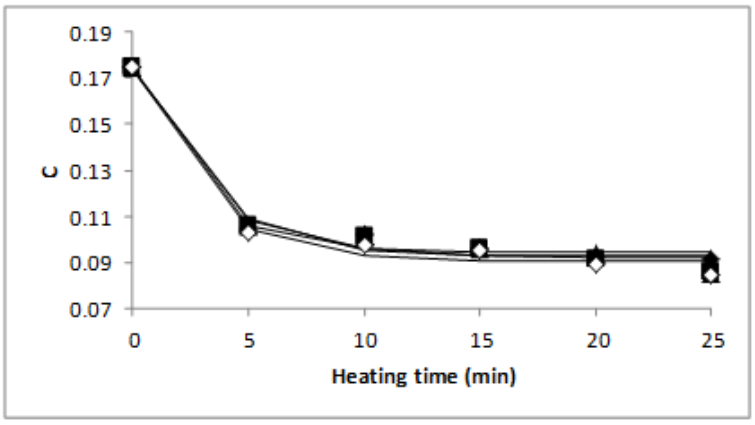
Temp °C	TAC		TPC		TFC		DPPH RSA	
	$k \cdot 10^{-2}$ min^{-1}	$t_{1/2}$	$k \cdot 10^{-2}$ min^{-1}	$t_{1/2}$	$k \cdot 10^{-2}$ min^{-1}	$t_{1/2}$	$k \cdot 10^{-2}$ min^{-1}	$t_{1/2}$
60	31.97 ±8.90	2.16 ±0.45	38.24 ±10.83	1.81 ±0.25	2.62 ±0.27	26.40 ±3.24	1.15 ±0.03	60.19 ±4.12
70	32.78 ±7.41	2.11 ±0.62	42.20 ±4.81	1.64 ±0.11	2.85 ±0.25	24.27 ±4.51	1.61 ±0.02	42.99 ±3.57
80	35.82 ±8.38	1.93 ±0.27	44.90 ±13.28	1.54 ±0.12	4.97 ±0.36	13.93 ±1.54	1.70 ±0.03	40.67 ±2.38
90	39.14 ±8.91	1.77 ±0.12	45.63 ±12.64	1.51 ±0.17	9.02 ±1.34	7.67 ±1.85	1.75 ±0.03	39.60 ±2.47
E_a kJ·mol^{-1}	6.95±1.22		5.99±1.20		42.50±9.42		13.35±5.01	

Jaiswal et al. (2012) used the zero-order, first-order and first-order fractional conversion kinetic models to assess the degradation rates of polyphenols, antioxidant activity and flavonoids from the Irish York cabbage at blanching temperature. Thus, at 80°C, the *k* value for the first order kinetic model was 0.149± 0.00 min^{-1}, whereas for the fractional pattern was 0.379±0.04 min^{-1}. In the temperature range of 80-100°C, the degradation rate constants increased gradually for both of the models, thus at 100°C a *k* value of 0.203 ± 0.02 min^{-1} was recorded

for 2nd order kinetic model, while in the case of the fractional model the *k* value was 0.484 ± 0.04 min^{-1}, with an activation energy of 11.54 kJ/mol.



(a)



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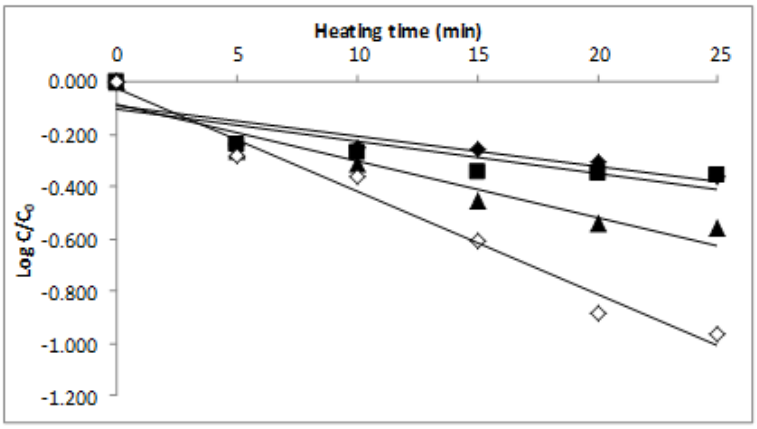

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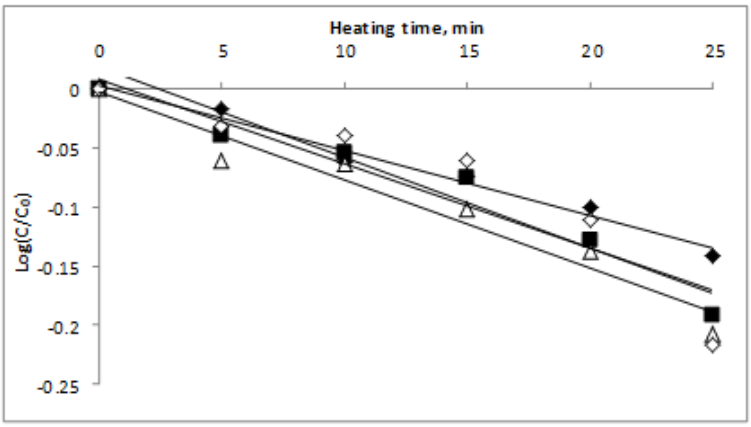

(d)

Figure 4. Fractional conversion and first-order plots for the degradation of TPC (a), TAC (b), TFC (c) and DPPH-RSA (d) from blackthorn extract during heating over the temperature range of 60-90°C for 0-25 min (♦60°C, ■ 70°C, ▲80°C and ◊90°C). The extract solutions were heat treated in a water bath. Data are the mean of triplicate samples.

The thermal degradation of anthocyanins from purple potato (Cv. Purple Majesty) followed the first-order kinetics model, with *k* values ranging from 0.0262 to 0.2855 min^{-1}, as suggested by Nayak et al. (2011). Significant higher k values were reported by Cao et al. (2011) for the thermal degradation of anthocyanins from blood orange juice, of 5064 min^{-1} at 70°C and increases at 14922 min^{-1} at 90°C. The thermal degradation of blackberry juice anthocyanins followed a first order reaction kinetics with respect to temperature, with *k* values increasing from $0.69 \cdot 10^3$ min^{-1} at 60°C to $3.94 \cdot 10^3$ min^{-1} at 90°C.

Thermal processing techniques used in the food industry may have an impact on the flavonoid structures, and therefore, influence their bioavailability and bioactivities (Rohn et al., 2007). Therefore, Pietta (2000) suggested that flavonol, flavone, and isoflavone glycosides usually require hydrolysis to their respective aglycons to become bioactive. From Table 1 one can see that no significant changes of the *k* values were found when the temperature increased from 60°C to 70°C. However, at higher temperature, a significant increase of the rate constant was observed. This behavior is completely different from anthocyanins, which are strongly affected by thermal treatment. Our results are in good agreement with those reported by Dhuique-Mayer et al. (2007) and Sanchez-Moreno et al. (2005), who suggested that pasteurization, did not modify the hesperetin content. These authors considered that this is due to the difference between the chemical structures of these molecules belonging to the subclasses of flavonoids, mainly based on the c-ring and particularly with the presence of the positive charge.

Turturică et al. (2016) reported an increase of the *k* values from $1.20 \pm 0.80 \cdot 10^{-2}$ 1/min at 70°C to $2.50 \pm 0.60 \cdot 10^{-2}$ 1/min at 110°C concerning the thermal degradation of TFC in plums extract. An increase of the *k values* from $13.60 \pm 2.00 \cdot 10^{-2}$ 1/min to $14.80 \pm 2.00 \cdot 10^{-2}$ 1/min with the temperature increasing

from 80°C to 90°C was reported by Jaiswal et al. (2012) for the thermal degradation of TFC in York sprouts.

No significant variations of the *k* values associated with the impact of heat treatment on the DPPH-RSA of blackthorn extract were observed (Table 1).

The half-life calculation results are presented in Table 1. TPC had the lowest half-life at 60°C, of only 1.81±0.25 min, while TFC and DPPH had the highest values of 26.40±3.24 min and 60.19±4.12 min, respectively. Heating the blackthorn extract caused a decrease of the half-lives, for all phytochemicals with the most significant decrease of the corresponding value for TFC (Table 1). Our results are significantly lower than those reported by Kopjar et al. (2009) and Kırca et al. (2007). For example, Kırca et al. (2007) suggested half-lives for the anthocyanin from black carrots during heating at 70, 80 and 90°C between 2.3 h and 25.1 h depending on temperature, pH and solid content. Kırca and Cemoroglu (2003) determined $t_{1/2}$ values ranging from 0.4 to 3.4 h depending on the solid content after the thermal degradation of blood orange anthocyanins.

To determine the effect of temperature on the studied parameters, the constants obtained from Eq. (1) and (2) were fitted to an Arrhenius-type equation for each of the studied kinetic models. As it can be seen from Table 1, E_a values for TAC and TPC were 6.95±1.22 and 5.99±1.20 kJ·mol^{-1}, significantly lower than those for TFC and DPPH-RSA (42.50±9.42 and 13.35±5.01 kJ·mol^{-1}, respectively). A higher E_a value caused a faster increase of the degradation rate when the temperature rose. This means that a higher E_a value demonstrated a higher stability of TFC at lower temperatures, while a faster degradation rate was registered at higher temperatures.

Liu et al. (2014) studied the degradation kinetics of anthocyanins from various fruits such as: apple, grapes, peaches, pears, lemons and grafts and reported a variation of the activation energies between 14.77 and 47.94 kJ/mol. A significantly higher value was reported by Harbourne et al. (2008) for the degradation of blackcurrant anthocyanins in a model juice heated over a temperature range of 21-100°C (73.0 ± 2.0 kJ/mol).

Sui et al. (2014) studied the thermal degradation of cyanidin-3-glucoside and cyanidin-3-rutinoside in the temperature range of 100-165°C, at pH 4.0, suggesting an activation energy of 94.4 kJ/mol and 90.5 kJ/mol, respectively. These authors established that the pH has a major influence on the stability of anthocyanins in addition to the heat treatment, reporting that the pH values less than 6.0 minimizes the effect of heat treatment. Cemeroglu et al. (1994) reported an E_a value of 68.52 kJ/mol for the anthocyanin's thermal degradation from cherry juice, whereas a higher value was suggested by Kırca and Cemoroglu (2003) in the case of blood orange juices (73.60 kJ/mol).

Conclusions

The results obtained from the present study provide detailed data regarding the heat induced changes in the phytochemicals content of blackthorn extract in relation to the antioxidant activity based on HPLC, fluorescence spectroscopy and kinetic modeling. Four anthocyanins were identified: cyanidin-3-glucoside, cyanidin-3-

rutinoside, peonidin-3-glucoside, peonidin-3-rutinoside and an unidentified compound. The results suggest that blackthorn has a good antioxidant activity, giving the possibility of using it as a food supplement or as an ingredient for functional foods.

Fluorescence spectroscopy technique and degradation kinetics were used to highlight the heat treatment effects on the blackthorn extract. The results revealed that the most effective fluorescence excitations of the extract were at the UV absorption maxima of 300 nm. However, heating the extract caused a decrease of the fluorescence intensity both when excited both at 270 nm and 300 nm. Significant red-and blue-shifts in the maximum emission induced by heating were found when excited at 270 nm, suggesting important structural degradation. The appearance of the chalcone forms of the anthocyanins was also suggested.

Degradation kinetics for the polyphenolic and anthocyanins content followed the fractional conversion kinetic model, while flavonoids and antioxidant activity showed a first-order reaction. The rate constants values showed that increasing of the temperature had accelerating effect on degradation. The results showed that polyphenolics degraded at a faster rate, whereas the antioxidant activity displayed the lowest *k* values. The activation energy values revealed a higher temperature dependence of flavonoids, followed by antioxidant activity, polyphenols and anthocyanins.

Advanced knowledge on the phytochemicals thermal degradation pattern based on their structure and functionality is needed in the food industry in order to optimize different technological processes, from the perspective of obtaining new and/or functional products.

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