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Effects of storage temperature on the total phenolic content of Cornelian Cherry (*Cornus mas* L.) fruits extracts

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Summary

Cornelian cherry (*Cornus mas* L.) fruits are an important source of bioactive compounds, especially phenolics and anthocyanins which exhibit a high antioxidant activity and contribute to their numerous health benefits. The aim of this study was to evaluate the effect of storage temperature on the stability of polyphenolic compounds of Cornelian cherry (*Cornus mas* L.) fruits extracts, stored under darkness at 2 °C, 22 °C, 55 °C and 75 °C respectively. For all analysed conditions, a first-order reaction kinetics was established for the degradation process of polyphenols. The degradation rate constants of the phenolic compounds at 2 °C and 22 °C presented comparable values (4.79 and 4.88 × 10^{-3} day^{-1}). The phenolics stored at 75 °C showed the lowest stability, with half-life value ($t_{1/2}$) and reaction rate constant (k) of 8.79 days and 78.76 × 10^{-3} day^{-1}, respectively. The temperature dependence of the polyphenols degradation rate constants was expressed by calculating the activation energy E_a (33.43 kJ mol^{-1}) and the temperature coefficients Q_{10} of the process. The Cornelian cherry (*Cornus mas* L.) fruits extract can be stored at least 2 months at room temperature without significant loss of their bioactive compounds.

Introduction

Fruits and vegetables are the main source of natural antioxidants, nutrients which play an important protective role against harmful free radicals which are responsible for cellular oxidation reactions and oxidative stress (Scalzo et al., 2005). Polyphenols and flavonoids are the most abundant dietary antioxidants (Aruoma, 1998).

The long term consumption of antioxidants-rich fruits plays an important role in human health by offering protection against oxidative stress related diseases, such as aging, vascular, heart and neurodegenerative diseases, cancer and diabetes (Smith et al., 2000; Tsuda et al., 2003;Vauzour et al., 2014).

Lately, the consumers' interest to particularly polyphenolic-rich fruits increased. Among these, Cornelian cherry fruits gained a great importance.

Cornelian cherry (*Cornus mas* L.) is a species of dogwood widely grown in Central and South-Eastern Europe and Asia. The deep red shinny fruit is spherical or oval shaped, one stone drupe, sweet-sour in taste but astringent when unripe. Cornelian cherries are edible fruits that can be eaten fresh, dried, pickled or used to produce different drinks, gels and jams. These fruits are rich in ascorbic acid (vitamin C), sugar, organic acids, flavonoids, tannins and other bioactive compounds among which polyphenols were found in significant amounts (David and Moldovan, 2015). These compounds, known for their high antioxidant activity, are responsible for the wide range of biological properties of these fruits, among which the antibacterial, anti-histamine, anti-inflammatory, anti-microbial and antimalarial properties were reported (Celik et al., 2006; Vareed et al., 2006). Due to all these health benefits, there is lately an increased interest on the physical and chemical properties, total phenolics and ascorbic acid content and antioxidant activity of Cornelian cherries (Pantelidis et al., 2007; Popovic et al., 2012).

Cornelian cherries extracts can be employed as functional ingredients in different nutraceutical products. So, the investigation of the thermal stability of the bioactive compounds of the extracts is of great interest. The characteristics and properties of the extracts must not be essentially affected by temperature elevation, which often occurs during industrial processing, or by storage conditions.

The effect of thermal treatments on fruit extracts and juices has been previously reported (Wang and Xu, 2007; Hillmann et al., 2011; Moldovan et al., 2011) and first order kinetics degradation was found for anthocyanins (Hernandez-Herreros and Frutos, 2011; Moldovan et al., 2012) while this tendency was not so clear for other polyphenolic compounds.

To the best of our knowledge, no kinetic data were published regarding the degradation kinetics of Cornelian cherry fruits polyphenols. The purpose of this study was to determine the stability of the polyphenols from Cornelian cherries extracts during storage at different temperatures. Therefore, the precise determination of the degradation kinetic parameters of the polyphenols present in these fruits is essential for predicting quality losses of nutritional values related to both storage and heat processing of foods. To these end, extracts were stored at 2 °C, 22 °C, 55 °C and 75 °C. The investigated temperatures were chosen in order to assure a high compatibility with processing techniques required to extend the shelf-life of food products, as thermal processing is the most effective treatment used to preserve food.

Material and methods

Plant material

Fruits of Cornelian cherry (*Cornus mas L.*) were obtained from a local market in Cluj-Napoca, Romania in August 2014. The specimen was identified and authenticated by Prof. Emeritus M. Tamas, Department of Botany, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj Napoca, Romania. 45 uniform ripened fruits at consume maturity stage were chosen. Skin colour (dark red) was the main criterion for maturity of fruits. The fruit mass ranged from 1.6 to 1.8 g with an average flesh: fruit ratio of 82%. Samples of 15 fruits in 3 replications were frozen and stored in polyethylene bags at -18 °C until used for the extraction of polyphenols.

Chemicals and reagents

All reagents and solvents were of analytical grade and purchased from Merck (Darmstadt, Germany). Distilled water was used for analyses and was obtained using a TYPDP1500 Water distiller (Techosklo LTD. Czech Republic).

Preparation of fruits extract

The stones of 15 frozen Cornelian cherries were removed and the fruits were milled. Ten grams of milled fruits were transferred to an Erlenmeyer flask and homogenised with 100 ml of cold acetone. The

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extraction was carried out for 1 h at room temperature. The obtained mixture was vacuum filtered through Whatman no. 1 paper. The acetone was totally removed under vacuum and a concentrated extract was obtained and further analyzed to determine the total phenolic content as well as the variation of this parameter in different storage conditions in order to establish the degradation kinetics.

Determination of total phenolic content (TPC)

The total phenolic content was assessed using the Folin-Ciocalteu reagent (SINGLETON et al., 1999). Six mL Folin-Ciocalteu reagent were mixed with 1 mL of diluted extract (dilution factor = 256) and allowed to react for 5 min in the dark. After that, 4.8 mL of Na_2CO_3 solution (0.7 M) were added and the resulted solution was kept in the dark for 2 h at room temperature (22 °C). The absorbance of the mixture was measured at 765 nm (using an UV-Vis Perkin Elmer Lambda 25 double beam spectrophotometer), against a blank sample. The total phenolic content was calculated with reference to a gallic acid (GA) calibration curve (range 0 - 100 mg mL^{-1}), and expressed as mg GA equivalents L^{-1} extract. Each measurement was performed in triplicate.

Degradation studies

The thermal degradation of the phenolic compounds from Cornelian cherry fruits extract was investigated at four different temperatures: 2 °C, 22 °C, 55 °C and 75 °C. The fruit extract was divided into 5 ml portions and kept in closed vials in order to prevent solvent evaporation. The samples were stored in the dark at 2 °C (in refrigerator) and at room temperature (22 °C) for 2 months, in a thermostatic water bath, preheated at 55 °C for 30 days and at 75 °C, respectively (± 1 °C) for 10 days. Samples were analyzed for the total phenolic content at 0, 10, 20, 30, 40, 50 and 60 days for storage at 2 and 22 °C, at 0, 5, 10, 15, 20, 25 and 30 days for storage at 55 °C and at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days for storage at 75 °C. Changes of the total phenol content were used to evaluate the stability of the polyphenolic compounds in the investigated extracts. All experiments were carried out in three replicates.

Data analysis

All data presented are mean values ± standard deviations of three independent experiments (n = 3). Data obtained were statistically analyzed by one-way variance analysis (ANOVA) using the statistical package XLSTAT Release 10 (Addinsoft, Paris, France). Statistical significances were considered acceptable at $p < 0.05$.

Results and discussion

The determined total phenolic content of the Cornelian cherry fruit extract was 1137.78 ± 21.44 mg L^{-1} expressed as gallic acid equivalents. The stability of polyphenols from the extract during storage at different temperatures (2 °C, 22 °C, 55 °C and 75 °C) was evaluated based on changes in their concentration. Tab. 1 shows the kinetic parameters (kinetic rate constants and the half-life values) determined for the thermal degradation of the polyphenolic compounds.

Tab. 1: Effect of temperature on the kinetic parameters of polyphenols degradation in Cornelian cherries extracts

Temperature/°C	$k \times 10^{-3}$/(day^{-1})*	$t_{1/2}$/days
2	4.79 (0.9379)	144.67
22	4.88 (0.9501)	142.01
55	38.86 (0.9693)	17.83
75	78.76 (0.9858)	8.79

*Numbers in parentheses, R^2, are the determination coefficients.

Previously reported data showed that storage and thermal degradation of polyphenols from various sources is described by simple first-order reaction kinetics (WANG et al., 2008; LI et al., 2012). The degradation kinetics of polyphenols can be described by following equation (1):

$$\ln[TPC] = \ln[TPC_0] - kt \quad (1)$$

where: $[TPC]$ = total phenolic content, mg L^{-1} at time t; $[TPC_0]$ = initial total phenolic content, mg L^{-1}; k = reaction rate constant, days^{-1}; t = reaction time, days.

The half-lifes of polyphenols from the investigated extracts during storage can be calculated using equation (2):

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

where: $t_{1/2}$ = half-life (days); k = reaction rate constant (days^{-1}).

According to equation (1) a series of k at different temperatures can be obtained by plotting the changes in the total polyphenolic content of the aqueous extract obtained from Cornelian cherries as a function of time (Fig. 1). High values of coefficients of determination (R^2, 0.93-0.98) were obtained for all linear regressions, confirming that the degradation process of these bioactive compounds, at temperatures from 2 °C to 75 °C, indeed followed first-order reaction kinetics.

The degradation of polyphenols from Cornelian cherries extract was investigated. Storage time and temperature had a significant effect ($p < 0.05$) on total phenolic content. During storage of Cornelian cherries extracts at 2 °C the level of phenolic compounds slightly decreased. We observed losses of 3.1 %, 6.9 %, 11.9 % and 22.6 % after 10, 20, 30 and 60 days of storage, respectively. Storage of extracts at room temperature (22 °C) resulted in no significant change of the degradation rate constant ($p = 0.9$), the lost of phenolic compounds being 25.4 % after 60 days of storage. Stability of polyphenolic compounds appears to be greatly affected at temperatures higher than 50 °C. In the case of thermal treatment on Cornelian cherry fruits extracts at 55 °C and 75 °C, significant decrease can be observed in total phenolic content values. This is consistent with results presented in previous studies on polyphenols degradation (KALT, 2005; KYI et al., 2005). The decrease of the polyphenolic content of the investigated extracts at high temperatures may be due to increased oxidation of these bioactive components. The rates of these oxidation reactions increase as temperature increases. The degradation rate of polyphenols stored at 55 °C was 8 times faster as compared to degradation at refrigerated storage (at 2 °C) while, at 75 °C, the degradation rate was 16 times higher (Tab. 1).

The activation energy normally describes the required energy to reach the transition state of a reaction. This energy can be determined from the kinetic rate constants by using an Arrhenius model (eq. 3):

$$k = K_0 e^{-Ea/RT} \quad (3)$$

where: k = rate constant, day^{-1}; K_0 = frequency factor, day^{-1}; E_a = activation energy, kJ mol^{-1}; R = universal gas constant (8.314 J mol^{-1} K^{-1}); T = absolute temperature, K.

Fig. 2 presents the plot of the degradation rate constants of the polyphenols from each extract as function of storage temperature. The slope of ln k *vs.* $1/T$ was used to determine the E_a value of the polyphenols degradation reactions.

The calculated E_a value was 33.43 kJ mol^{-1} ($R^2 = 0.915$), comparable to that determined by other authors, who reported a value of 30.324

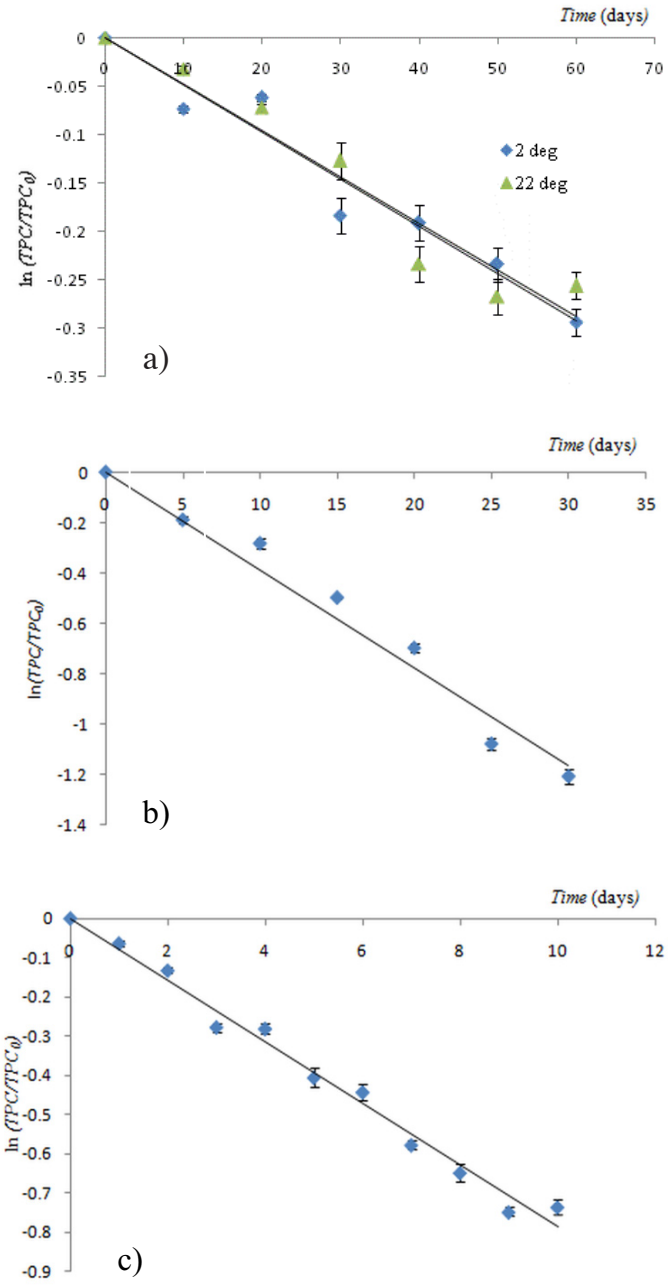

Fig. 1: Degradation kinetics of polyphenols during storage at (a) 2 °C and 22 °C; (b) 55 °C; (c) 75 °C (vertical lines represent SD, n = 3)

kJ mol^{-1} for the degradation of polyphenols from cocoa beans (KYI et al., 2005).

The temperature coefficient Q_{10} is another parameter which, in addition to activation energy, provides information related to temperature influence on the degradation rate of polyphenols. Q_{10}, defined as the change of degradation rate upon a temperature increase of 10 K, can be calculated by the following equation (4) (MOLDOVAN and DAVID, 2014):

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2 - T_1)} \quad (4)$$

where: Q_{10} = the temperature coefficient, K^{-1}; $k_{1,2}$ = rate constant, day^{-1} at temperature $T_{1,2}$, K.

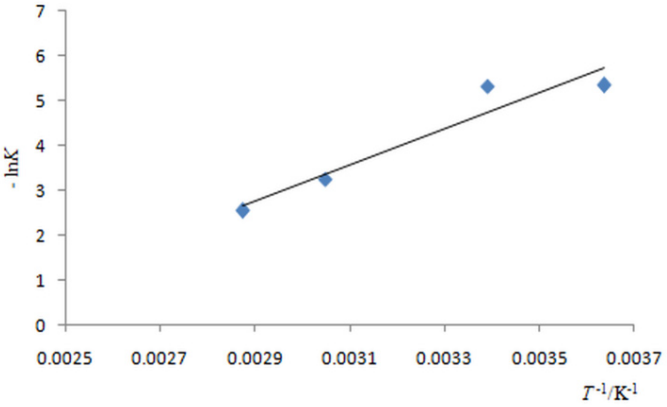

Fig. 2: Arrhenius plot for polyphenols degradation in Cornelian cherries extracts

Tab. 2 presents the Q_{10} values calculated for the temperatures applied in this study.

Tab. 2: Q_{10} and E_a values of polyphenols' degradation at different storage temperatures

E_a/kJ mol^{-1}*	K_o/day^{-1}	Q_{10}		
		2-22 °C	22-55 °C	55-75 °C
33.43 (0.915)	$7.39 \cdot 10^3$	1.009	1.875	1.423

*Number in parentheses, R^2, is the determination coefficient.

The highest Q_{10} value (Q_{10} = 1.875) was obtained for storage at temperatures between 22 and 55 °C indicating that degradation of phenolics was stronger affected by temperature within the range 22-55 °C than between 55-75 °C (Q_{10} = 1.423). The lowest temperature coefficient value (Q_{10} = 1.009), determined for storage temperatures 2-22 °C, proves that increasing temperature from 2 °C to 22 °C has no influence on the degradation process of these bioactive compounds from Cornelian cherries extracts (Tab. 2).

The results obtained in this study regarding the degradation kinetics parameters of the polyphenols from Cornelian cherry fruits extracts are comparable to the literature data reported for the degradation process of phenolics from other sources such as *Hibiscus* and cocoa beans, although the degradation process of the total phenolics from fruits was less investigated. PEREZ-RAMIREZ et al. (2015) reported a half-life value of 17.2 days for the degradation of the polyphenols from *Hibiscus sabdariffa* L. beverages stored at 50 °C, value in agreement with our results ($t_{1/2}$ = 17.8 days at 55 °C).

Comparing the loss of total phenolics from Cornelian cherry fruits extracts during thermal processing and storage to that of anthocyanins isolated from the same fruits, extensive anthocyanin degradation occurred under the same heating and storage conditions. Previous studies (MOLDOVAN and DAVID, 2014) reported accelerated anthocyanin degradation, the half-life values for this process being 2.4 fold higher at 2 °C, 4.3 fold higher at 22 °C and 25 fold higher at 75 °C, as compared to degradation of total phenolics, indicating a higher stability of Cornelian cherry polyphenols. This fact may be related to the degradation pathway of Cornelian cherry anthocyanins. The major anthocyanins in Cornelian cherries are cyanidin-3-O-galactoside, pelargonidin-3-O-galactoside and delphinidin-3-O-galactoside (VAREED et al., 2006). The A and B rings of these anthocyanins can degrade into other phenolic derivatives, such as *p*-hydroxibenzoic acid, protocatechuic acid or phloroglucinaldehyde (SADILOVA et al., 2007).

Conclusions

The present study evaluated the influence of the temperature and storage on the degradation kinetic parameters of phenolics from Cornelian cherries extracts. Phenol content was stable at temperatures up to 22 °C. Temperature increase determined higher rate constants of the degradation process. The extracts can be stored during 2 months at room temperature, without significant decrease of their total phenolic content. The bioactive compounds made responsible for the health promoting effects of Cornelian cherry fruits were not substantially affected even after excessive long time heating. It should be highlighted that Cornelian cherry fruits could be an interesting source of nutraceuticals, considering the high stability to storage and temperature of their nutritional parameters.

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