KINETIC STUDY OF OXIDATION DEGRADATION OF POLYPHENOLS IN SOUR CHERRY AND BLACKBERRY EXTRACTS DURING STORAGE

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Abstract

In this study was to investigate the influence of storage time, light and temperature on stability of polyphenols in sour cherry (*Prunus cerasus*) and blackberry (*Rubus fruticosus*), were harvested in western Serbia (Rasinski region). Total phenol content was monitored in the fruit extracts during 23 days stored at 7°C under darkness and 90 days storage at 23°C in oxygen. For analyzed extracts, first-order reaction kinetics was established for the degradation process of polyphenols. The temperature dependence of the polyphenols degradation rate constants was expressed by the temperature coefficients Q_{10} of the process. It is found that the Q_{10} values of polyphenols degradation in sour cherry and blackberry extracts were 1.247 and 3.239, respectively.

Keywords: Sour cherry, Blackberry, Polyphenols, Storage Time, Temperature

Introduction

Epidemiological studies have indicated that frequent consumption of fruits and vegetables is associated with a lower risk of cardiovascular disease and cancer (Hou, 2003). Fruits are important source of bioactive compounds, especially polyphenols which play an important protective role against harmful free radicals, which are responsible for cellular oxidation reactions and oxidation stress (Rufino et al., 2009). Over 8000 phenolic compounds have been identified from plant materials and they possesses a wide spectrum of biochemical activities (antioxidant, antimicrobial, antimutagenic, anticancerogenic and ability to modify the gene expression) (Jakobek et al., 2007).

The major sources of polyphenols in edible plants are families *Vitaceae* (grape) and *Rosaceae* (cherry, plum, raspberry, strawberry, blackberry, apple, peach, etc.). As polyphenols as antioxidants are becoming an important parameter with respect to fruit quality, it is of great interest to evaluate changes in total phenolic content during

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temperature storage of fruit extracts, which have potential pharmaceutical application. During processing and subsequent storage changes can occur such as thermal degradation of polyphenols (Cemeroglu al., 1994; Garcia-Viguera et al., 1999; Ochoa et al., 1999). This study was undertaken to investigated the influence of different temperatures on polyphenol stability of extracts of sour cherry (*Prunus cerasus*) and blackberry (*Rubus fruticosus)*, originally from western Serbia (Rasinski region).

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH˙) in free radical form was obtained from Sigma Chemical Co. (St. Louis, MO). Methanol, gallic acid, catechin and quercetin were purchased from Merck Co (Germany). All reagents were analytical grade.

Plant materials

Samples of sour cherry (*Prunus cerasus)* and blackberry (*Rubus fruticosus)*, were harvested in western Serbia (Rasinski region) at the commercial maturity stage in June-August 2014.

Preparation of extracts

Fruit extracts were obtained by grinding berries (10 g) in 100 mL methanol/water solution (70/30) for 30 min at room temperature. The mixture was stored at 7°C in the dark for 24^h and then centrifugated at 4000 rpm for 15 minutes. Extracts were purified through a 0.45 μm syringe filter (Millipore) before analyses.

Determination of phenolic composition

The amount of total phenol content, hydroxycinnamoyl tartaric cids and yotal flavonols in fruit extracts was determined according to the Mazza and Miniati (1993) procedure: 0.25 mL of extract and 0.25mL of 0.1% HCl in 95% ethanol were mixed with 4.55 mL of 2% HCl and absorbance was measured at 280 nm with a UV/VIS Agilent 8453 spectrophotometer, after 15 min incubation at the room temperature. Total phenol content was expressed as mg of gallic acid equivalents/100g of fruit, mg of caffeic acid equivalents/100g of fruit for hydroxycinnamoyl tartaric cids and mg of quercetin equivalents/100 g fruit for total flavonols.

Determination of monomeric anthocyanins

The total monomeric anthocyanin content in fruit extracts was determined with the pH-differential absorbance method described by Guisti and Wrolstad (2001). Anthocyanins have maximum apsorbance at a wavelenght of 513 nm at pH of 1.0. The coloured oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. Absorbance of the fruit extract was measured at 520 i 700 nm in potasium chloride buffer (pH 1) and sodium acetat buffer (pH 4.5) after 15 min incubation at the room temperature. Anthocyanin content was expressed in mg of cyanidin-3-glucoside equivalent (Cygl)/100g fruit using a molar extinction coefficient of cyanidin-3-glucoside of 26900 L/molcm and molar weight 449.2 g/mol.

Determination of indices for anthocyanin pigment degradation, polimeric colour and browning

Indices for anthocyanin degradation of the fruit extracts can be derived using the pH-differential method described by Giusti and Wrolstad 92001). The absorbance at 420 nm of the disulphide treated sample serves as an index for browning. The color density of the control sample and the polymer color of the disulphide bleached sample are calculated as follows:

Color density = $[(A_{420nm} - A_{700nm}) + (A\lambda_{max} - A_{700nm})]$

The value of hue is calculated as follows:

Hue = $[(A_{420nm} - A_{700nm})/ (A\lambda_{max} - A_{700nm})]$

And the ratio between polymeration color and color density is used to determine the percentage of the color that is contributed by polymerized material.

Antioxidant activity

The antioxidant capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH▪) scavenging assays (Wang and Lin, 2000). The antioxidant assay is based on the measurement of the loss of DPPH˙ colour by the change of absorbance at 517 nm caused by the reactions of DPPH˙with the tested samples. Reaction solution was prepared by mixing 2.5 mL of diluted fruit extract with 1 mL of methanolic DPPH solution. The solution was kept in dark at room temperature for 20 min. Scavenging capacity of DPPH• in percent (%) of each fruit extracts sample was calculated from the decrease of absorbance according to the relationship:

Antioxidant capacity (%) = $(1 - A_{sample} - A_{blank}/A_{control})$ x 100

Where $A_{control}$ is the absorbance of control reaction, A_{blank} is the absorbance of diluted fruit extract sample and A_{sample} is the absorbance of the diluted fruit extract sample with DPPH radical. The antioxidant activity (%) was plotted against the plant extract concentration (mg/mL) to determine the concentration of extract that reduces activity by 50% (EC_{50}).

Degradation studies

The thermal degradation of the total phenol content from investigated fruits extract was investigated at 7 °C during 23 days in dark and 90 days storage at 23°C in air. One part of extracts was kept in glass bottles, protected from light and refrigerate up to 7 ^oC and a second part of samples was exposed to daylight at room temperature (23^oC) in laboratory conditions. Samples were analyzed for the total phenolic content at 0, 1, 4, 23, 57 and 90 days. Changes of the total phenol content were used to evaluate the stability of the polyphenols in the investigated extracts. All experiments were carried out in three replicates.

Kinetic Modeling

Kinetic modeling of experimental data was performed with software GraphPad Prism version 5.00 for window, using the general equations of for a first –order, as follows:

$lnCt = lnC_0 - kt$

where Ct is total phenol concentration at time t (mg/100g fw), C_0 is initial total phenol concentration at time 0 (mg/100g fw), t is the time (days), and k is the constant of reaction (expressed mg/ day).

Statistical analysis

Measurements were averaged, and results are given as mean ± standard deviation (SD). One-way ANOVA was used to determine differences between measurements. Differences at $p<0.005$ were considered to be significant.

Results and discussion

The composition of phenolic compounds in fruits is influenced by genotype, storage conditions, extraction procedure, and environmental conditions. The spectrophotometric assays were performed for simply determination of phenolic composition in sour cherry (*Prunus cerasus*) and blackberry (*Rubus fruticosus)* extracts, and the results are shown in Table 1:

Table 1. *Concentrations of total phenols, hydroxycinnamoyl tartaric esters, flavonols, monomeric anthocyanins (mg/100 g fruit), polymeric color (%) and antioxidant activity, EC_{50} (mg/mL) in investigated fruit extracts*

	Sour cherry extract	**Blackberry extract**
Total phenols	273.22 ± 4.38	230.00 ± 1.09
Hydroxycinnamoyl tartaric esters	44.42 ± 2.45	24.81 ± 2.23
Flavonols	24.42 ± 2.38	18.06 ± 2.83
Monomeric anthocyanins	121.95 ± 1.37	84.16 ± 2.82
Polymeric color	21.14 ± 0.72	12.20 ± 0.32
Antioxidant activity	0.52 ± 0.01	0.59 ± 0.01

Similar results for phenolic composition in fruit extracts have been reported by other authors (Pantelidis et al., 2007). It has been found that concentrations total phenols, hydroxycinnamoyl tartaric esters, flavonols and anthocyanins of sour cherry extracts are higher than in raspberry extracts. Data presented by other researchers from neighboring countries in blackberry are similar to the results for anthocyanin content we have been investigated (Marinova et al., 2005; Jakobek et al., 2007). Polymeric colour has a lower value in blackberry (12.20%) compared with the sour cherry extract (21.14%). In order to evaluate antioxidant activity of fruits, DPPH assay was applied. Analyzed fruit extracts exhibited significant antioxidant activity (Table 1). The sour cherry extracts have higher antioxidant activity (0.52 mg/mL) than the extracts of raspberry (0.59 mg/mL), which is in accordance with their phenolic contents.

In the literature it has been reported the effect of storage temperature in pasteurized fruit juices. Oliveira et al. (2014) reported 49 % decrease in total phenolics in pasteurized strawberry during storage at 23 ^{o}C for 90 days followed a zero-order kinetic model. It has been reported also, that the freezing process decreased the total phenolic content and antioxidant capacity by 4-20 % in raspberries juice (Piljac-Zegavac et al., 2009). In this present study investigate the change in total phenol content during storage of different temperatures and time of selected fruit extracts. One group of fruit extracts were kept at 7 °C during 23 days in dark, and second group were hold at room

temperature (23 °C), exposed to sun light, during 90 days. The changes of total phenol contents in these samples are shown in Table 2 and 3:

***Table 2**. The changes of total phenol content in investigated fruit extracts (mg GAE /100g fruit) at 7°C during 23 days in dark*

Time storage	Sour cherry extract	Blackberry extract
1 day	273.22 ± 4.38	230.00 ± 1.09
4 day	275.04 ± 3.25	231.13 ± 2.65
23 day	265.76 ± 1.59	228.47 ± 2.13

After stabilization of the extracts, the first 4 days there was a slight increase in the concentration of polyphenols by 0.5%, which was followed with a slight decrease in the further course of storage. The decreases of phenolic concentrations in sour cherry and blackberry extracts after 23 days at 7°C were 2.73% and 0.66%, and after 90-days storage at 23°C were 14.93% and 13.69%, respectively. These changes the content is found in the works of other (Tsud et al., 2000; Jakobek al., 2007; Li et al., 2012). The decrease in total content of investigated fruit extracts after 23 day was not statistically significant. On the other hand, total phenol content in selected fruit extracts stored at room temperature (23 ^{o}C) and exposed to sun light was increase after 4 days, and was followed by a decrease of these values after 90 days storage (Table 3):

***Table 3**. The changes of total phenol content in investigated fruit extracts (mg GAE /100 g fruit) at 25°C, exposed to sun light, during 90 days*

Time storage	Sour cherry extract	Blackberry extract
1. day	273.22 ± 4.38	230.00 ± 1.09
4 day	275.15 ± 2.45	235.52 ± 2.23
23. day	270.78 ± 2.38	211.28 ± 2.83
57 day	259.19 ± 1.37	200.12 ± 2.82
90 day	232.43 ± 1.72	198.50 ± 3.32

Application of kinetic models in foods facilitates the assessment and prediction of the influence of processing operations and parameters on critical quality attributes, in order to minimize undesirable changes and to optimize quality (Jorgensen et al., 2004; Patras et al., 2010). Degradation is primarily caused by oxidation, cleavage of covalent bonds, or enhanced oxidation reactions due to thermal processing. Studies on the change of individual phenolic concentrations, especially anthocyanins, adopted first-order kinetic models (Wang and Xu, 2007; Alighouchi and Barzegar, 2009). Table 4 shows the reaction rate constants (k) and the half-life values ($t_{1/2}$) for the degradation of the total phenols, during storage on different temperatures. The half-lifes of phenols during storage can be calculated using follows Eq.

$t_{1/2} = -ln\ 0,5/k$

Table 4. *Effect of temperature on the kinetic parameters of total phenol degradation in analyzed fruit extracts*

Fruit extracts	**7°C**			**23°C**		
	kx10^{-3}(day^{-1})	**$t_{1/2}$ (day)**	**R^2**	**kx10^{-3}(day^{-1})**	**$t_{1/2}$ (day)**	**R^2**
Sour cherry	1.450	478.03	0.95	2.02	343.14	0.97
Blackberry	0.412	1684.03	0.85	2.40	288.81	0.84

Temperature in the presence of oxygen can also induce degradation of proanthocyanidins into their flavan-3-ols or covalently bound to phenolic compounds; leading to an increase of soluble phenolics (Rawson et al., 2011) reported temperature treatments favor the rupture of cellular structures increasing the exposure of substrates to nonenzymatic oxidations. This phenomenon is one of the main reasons for the loss of phenolic compounds.

High values of determination coefficients, R^2, confirming that degradation of total phenols from investigated fruit extracts during storage follows first-order kinetic model. In the literature, the storage degradation of polyphenols from different sources is also described by first-order reaction kinetics (Wang et al., 2008; Li et al., 2012). The temperature coefficient Q_{10} (K^{-1}) defined the change of polyphenols degradation rate upon 10K temperature increase. It can be calculated using follows Eq :

$Q_{10} = (k_2/k_1)^{10/(T2-T1)}$, where $k_{1,2}$ are rate constants at $T_{1,2}$ temperature (K) (Moldovan et al., 2016).

It is found that the Q_{10} values of polyphenols degradation in sour cherry and blackberry extracts were 1.247 and 3.239, respectively. Determined Q_{10} values indicate that increasing temperature from 7°C to 23°C has minimum influence on strawberry polyphenols degradation, while maximum effect is obtained for degradation process of blackberry polyphenols.

Conclusions

The result of the present study showed that sour cherry (*Prunus cerasus*) *and* blackberry (*Rubus fruticosus*) extracts are good sources of polyphenols. The fruit extracts exhibited fluctuations in total phenol content with an initial increase after 4 days, followed by a decrease in total phenol values at storage temperature. Decreases in the content of polyphenols of fruit extracts during 23 days storage at 7 °C in dark were not statistically significant, whereas significant variations were observed during storage at 23 °C during 90 days in air. The reduction of total phenols may be attributed to the light and room temperature transmission of glass bottles, which has oxidative affects on polyphenols. These results indicate that polyphenols in analyzed fruit extracts greater stability at 7 °C in dark compared to 23 °C in light and can be used as antioxidants, considering the high stability to storage and temperature.

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