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Kinetics of the Degradation of Anthocyanins, Phenolic Acids and Flavonols During Heat Treatments of Freeze-Dried Sour Cherry Marasca Paste

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Summary

The effect of heating temperature (80–120 °C) and processing time (5–50 min) on the stability of anthocyanins (cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside and cyanidin-3-glucoside), quercetin-3-glucoside and phenolic acids (chlorogenic, neochlorogenic, *p*-coumaric and ferulic acids) in freeze-dried Marasca sour cherry pastes was studied. The degradation rates of individual anthocyanins, quercetin-3-glucoside and phenolic acids followed the first order reaction kinetics. Cyanidin-3-glucoside was found to be the most unstable among the anthocyanins, together with *p*-coumaric and neochlorogenic acids among other phenols. Activation energies for anthocyanin degradation ranged from 42 (cyanidin-3-glucosylrutinoside) to 55 kJ/mol (cyanidin-3-glucoside), and for other phenols from 8.12 (chlorogenic acid) to 27 kJ/mol (neochlorogenic acid). By increasing the temperature from 80 to 120 °C, the reaction rate constant of cyanidin-3-glucosylrutinoside increased from $2.2 \cdot 10^{-2}$ to $8.5 \cdot 10^{-2}$ min^{-1}, of *p*-coumaric acid from $1.12 \cdot 10^{-2}$ to $2.5 \cdot 10^{-2}$ min^{-1} and of quercetin-3-glucoside from $1.5 \cdot 10^{-2}$ to $2.6 \cdot 10^{-2}$ min^{-1}. The obtained results demonstrate that at 80 °C the half-life of anthocyanins ranges from 32.10 min for cyanidin-3-glucosylrutinoside to 45.69 min for cyanidin-3-rutinoside, and of other phenolic compounds from 43.39 for neochlorogenic acid to 66.99 min for chlorogenic acid. The results show that the heating temperature and duration affect the anthocyanins considerably more than the other phenols in terms of degradation.

Key words: anthocyanins, phenolic acids, flavonol glycoside, freeze-drying, thermal degradation, sour cherry, *Prunus cerasus* var. Marasca

Introduction

Plant phenols comprise a great diversity of compounds, among which flavonoids and several classes of nonflavonoids are usually distinguished. They show a great diversity of structures, ranging from rather simple molecules to complex polymers (simple phenols, phenolic acids, flavonoids, stilbenes, coumarins, isocoumarins, tannins, *etc.*) (*1*). Phenolic acids can be divided into derivatives of benzoic acid and derivatives of cinnamic acid (*2*). Anthocyanins are one of the numerous subgroups of flavonoids, and are water-soluble natural pigments responsible for the blue, purple, violet and red colour of many fruits, vegetables, as well as their products (*3*). Phenols are biologically active compounds (BACs) with strong antioxidant capacity (*4,5*), so numerous researchers reported about their capacity of reducing the risk of coronary heart diseases, cancer and several

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chronic diseases (*6–8*). The major sources of anthocyanins, flavonol glycosides and phenolic acids in edible plants are blueberry, aronia, sour cherry, cherry, raspberry, blackberry, strawberry, plum, black currants, purple grapes, *etc*. (*9*). Among numerous fruit species, sour cherry Marasca (*Prunus cerasus* var. Marasca) is a rich source of polyphenols, especially anthocyanins, which contribute to its intense dark red colour (*10,11*). Because of the specific physicochemical properties, high dry matter content (21–27.3 %) (*12*), specific aroma and presence of bioactive compounds, it is an excellent raw material for processing into dry, frozen or freeze-dried cherries, jams, soft and alcoholic drinks, juice, concentrate and powder. Compared to other drying methods (conventional, convective air, vacuum oven, microconvection, solar drying, *etc*.) (*13*), freeze-drying is the optimal method for the production of high quality dehydrated fruit products. Due to the minimal degradation of BACs and microbiological stability, freeze-dried sour cherry Marasca retains sensory properties of fresh fruit and therefore can be used in the production of various functional products (*14–16*).

Stability of anthocyanins as the most unstable group of polyphenols mostly depends on processing and storage conditions (pressure, temperature, water activity, process duration, pH, light, oxygen, metal ions, enzymes and sugars) (*17–21*). Thermal treatments and storage temperature have the most important influence on anthocyanin stability (*17,22–24*). As reported by Asafi and Cemeroğlu (*25*), the rate of anthocyanin degradation in sour cherry and pomegranate juices and their concentrates during storage increased with the increase of temperature, and was higher in pomegranate samples.

According to Kopjar *et al*. (*26*), storage of sour cherry puree extracts at room temperature and at 4 °C for 42 days decreased the content of anthocyanins, total phenols and flavonoids. Fracassetti *et al*. (*27*) observed the thermal degradation of individual and total anthocyanins in freeze-dried wild blueberry powder, stored at temperature range of 25–80 °C for 49 days. They concluded that the degradation was significantly faster at higher temperatures. Considerable anthocyanin degradation occurred in the rubbery state of freeze-dried raspberries at selected water activities during one year of storage (*28*). There are numerous studies that describe the degradation of anthocyanins in different substrates, but only limited information is available about the stability of phenolic compounds and anthocyanins in freeze-dried cherry paste.

The aim of this research is to study the kinetics of individual anthocyanins, phenolic acids and flavonol glycosides in freeze-dried sour cherry Marasca paste, in order to advance the knowledge of the thermal stability of the above-mentioned compounds, and to establish mathematical models enabling the prediction of the degradation of these compounds during storage and thermal processing.

Materials and Methods

Sour cherry fruits

Sour cherry fruits (*Prunus cerasus* var. Marasca) were harvested at the plantation of Maraska factory (Zadar, Croatia) at technological stage of maturity during June 2011. After harvesting, the samples were immediately transported to a laboratory in a portable refrigerator at 4 °C, subsequently pitted, frozen using liquid nitrogen, and kept at –60 °C (ScanCool SCL210P, LaboGene™, Lynge, Denmark) until freeze-drying.

Freeze-drying of sour cherries

For freeze-drying of pitted sour cherries (previously frozen at –60 °C), 500 g of the sample were placed on each tray. Six trays were placed in a laboratory freeze--dryer (CoolSafe PRO, LaboGene™) and the freeze-drying process was performed for 24 h under high vacuum (13–55 Pa), with isothermal (heating) plate temperatures of 20 °C. The final water content of dried fruits was 9.7 %. Freeze-dried sour cherries were blended and homogenized in a paste with a house blender (Clatronic, Kempen, Germany) and immediately underwent heat treatment.

Heat treatment of freeze-dried sour cherry

Samples (homogenised freeze-dried sour cherry paste, approx. 5 g) were put in seven test tubes in inert nitrogen gas atmosphere and heated in an oil bath preheated to a given temperature: 80, 90, 100, 110 and 120 °C. Temperature of the oil bath was measured by glass thermometer (Amarell GmbH & Co. KG, Kreuzwertheim, Germany). Samples were taken after 5, 10, 15, 20, 30, 40 and 50 min. After the treatment, the tubes were cooled down in an ice bath to stop further thermal degradation. All heating treatments were done in duplicate and were followed by the extraction of polyphenolic compounds for further analysis.

Extraction of phenols

The extraction of phenols from heat-treated freeze--dried sour cherry was performed according to a previously described procedure (*29*). Phenols were extracted from heat-treated cherry samples ((2±0.001) g) with 8 mL of 80 % aqueous methanol solution containing 0.1 % HCl (by volume), in a water bath at 60 °C for 20 min. Afterwards, the extracts were filtered through Whatman No. 40 filter paper (Whatman, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), transferred into 10-mL volumetric flasks, and made up to volume with extraction solvent. Each sample was heat treated in parallel, and extracted twice (N=4). Extracts were stored at –60 °C in an inert nitrogen gas atmosphere before the analysis.

HPLC analysis

The anthocyanins, hydroxycinnamic acids and flavonol glycosides were simultaneously analysed by a direct injection of the extracts, previously filtered through a 0.45-μm pore size membrane filter (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Chromatographic separation was performed using HPLC analysis with Agilent 1260 Infinity quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with diode array detector (DAD), an automatic injector and ChemStation software.

The separation of phenolic compounds (anthocyanins, hydroxycinnamic acid and flavonol glycosides) was per-

formed on a Nucleosil 100-5C18, 5 μm (250 mm×4.6 mm i.d.) column (Macherey-Nagel). The solvent composition and the gradient conditions used were as described previously by Mitić *et al.* (*30*), with some modifications: instead of 5 % the solvents contained 3 % of formic acid.

For gradient elution, mobile phase A contained 3 % of formic acid in water, while solution B contained 3 % of formic acid in 80 % acetonitrile. The used elution program was as follows: from 0 to 28 min 0 % B, from 28 to 35 min 25 % B, from 35 to 40 min 50 % B, from 40 to 45 min 80 % B, and finally for the last 10 min again 0 % B. The flow rate was 0.8 mL/min and the injection volume was 5 μL.

Detection was performed with UV/VIS–photo diode array detector by scanning from 220 to 570 nm. Identification of phenols was carried out by comparing retention times and spectral data with those of the authentic standards (anthocyanins were identified at 520 nm, phenolic acids at 280 nm and flavonol glycoside at 360 nm).

The quantifications of anthocyanins, flavonol glycoside and phenolic acids were made by the external standard method. All anthocyanin standards, cyanidin-3--glucoside (Cy-3-G), cyanidin-3-rutinoside (Cy-3-R) and cyanidin-3-sophoroiside (Cy-3-S), were prepared as stock solutions in acidified methanol (1 % of formic acid in methanol, by volume) at a concentration of 100 mg/L. Working cyanidin standard solutions were prepared by diluting the stock solution to yield five concentrations in a range from 16.67 to 100 mg/L. Quantitative determination was carried out using the calibration curves of the standards Cy-3-G: y=21.952x, R^2=0.99; Cy-3-R: y= 14.969x, R^2=0.99; and Cy-3-S: y=13.355x, R^2=0.99. All identified anthocyanins were cyanidin derivatives differing in sugar moiety bonded to the aglyconic part of molecule. For the anthocyanin lacking reference standards, cyanidin-3-glucosylrutinoside (Cy-3-GR) identification was done in comparison with Cy-3-G.

Standards of phenolic acids and quercetin-3-glucoside (Q-3-G) were prepared as stock solutions in ethanol (ethanol/water=80:20 %, by volume) at following concentrations: chlorogenic acid (ChA) and caffeic acid (CA) 52 mg/L, *p*-coumaric acid (*p*-CA) 48 mg/L, ferulic acid (FA) 46 mg/L and Q-3-G 400 mg/L. Working standard solutions were prepared by diluting the stock solution to yield five concentrations in the range from 10.4 to 52 mg/L for ChA and CA, from 9.6 to 48 mg/L for *p*-CA, from 9.2 to 46 mg/L for FA, and from 80 to 400 mg/L for Q-3-G. Quantitative determination was carried out using the calibration curves of the standards (ChA: y= 14.571x, R^2=0.99; *p*-CA: y=10.729x, R^2=0.99; FA: y=19.291x, R^2=0.99; Q-3-G: y=10.79x; R^2=0.99; CA: y=21.294x, R^2= 0.99). For neochlorogenic acid (NChA) lacking reference standard, identification was done in comparison with ChA.

Identification was made by matching the retention time of the separated peaks and the retention time of the authentic standards. Additionally, identification was confirmed using characteristic UV/VIS spectra, polarity, previous literature reports (*30–33*), and also in another study on sour cherry Marasca products done with LC--MS/MS (results not shown).

Quantitative determination was based on peak area from HPLC analyses and from the mass fraction of the compound. The results were expressed as mg per 100 g of sample, as mean values±standard deviations (N=4 replicates).

Data analysis

The anthocyanin, phenolic acid and Q-3-G contents in freeze-dried sour cherry during heating treatment were plotted as a function of time. Previous studies had shown that thermal degradation of anthocyanins followed a first-order reaction kinetics (*34–38*). This kinetic type can generally be expressed using Eq. 1:

$$w(t)=w_0\cdot\exp(-k\cdot t) \qquad /1/$$

with $w(t)$ as the mass fraction at time t, w_0 as the initial mass fraction (μg per 100 g of sample), t as the treatment time (min) and k as the first order degradation rate constant (min^{-1}).

The half-life ($t_{1/2}$) of the reaction was calculated assuming the first-order kinetics as:

$$t_{1/2}=-\ln 0.5/k \qquad /2/$$

The temperature dependence degradation rate constant can be expressed with the Arrhenius equation:

$$k(T)=k_{refT}\cdot\exp\left[\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \qquad /3/$$

in which the temperature dependence of k is quantified by the activation energy E_a (kJ/mol) according to Eq. 2, k is the degradation rate constant at temperature T (K), k_{refT} is the degradation rate constant at temperature T_{ref} (383.15 K or 110 °C) and R is the universal gas constant ($8.314\cdot10^{-3}$ kJ/(K·mol)).

After integration of mass fraction as a function of time, temperature can be analytically derived for each isothermal experiment. Series of isothermal experimental data for each anthocyanin and other polyphenolic compounds are pulled together by series of isothermal integrations into a single function of two variables of temperature and time (Eq. 4), which are represented as projections of all the experimental data to a single surface in 3D plot.

$$\ln w(t,T)=-\ln w_0\cdot k_{refT}\cdot\exp\left[\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\cdot t \qquad /4/$$

Results and Discussion

In the presented research, the major anthocyanins determined in freeze-dried sour cherries were Cy-3-GR, Cy-3-R and Cy-3-G, whereas Cy-3-S was detected in traces. Their mass fractions were 73.55, 21.80 and 4.65 % of the total anthocyanin content, respectively. The determined phenolic acids were ChA, NChA, *p*-CA, FA and Q-3-G from a subgroup of flavonol glycosides. Their mass fractions were 33.46, 28.66, 26.58, 1.12 and 10.18 % of the total non-anthocyanin content, respectively. The quality of dehydrated foods is dependent on the changes occurring during processing and storage, and it is well known that freeze-drying produces the highest quality dried food with very high retention of BACs. According to Piasecka *et al.* (*39*), the stability of phenols was much

higher in freeze-dried sour cherries and other fruits than in convectively dried fruits. Temperature is one of the most critical factors that affect product quality and shelf--life. In general, in literature, the effects of heating on the degradation of biologically active compounds have been assessed in terms of total anthocyanins, rather than changes in the levels of particular anthocyanins and other phenolics.

The content of anthocyanins and other phenolics in freeze-dried Marasca sour cherries during heating at referent temperature of 110 °C was plotted as a function of time and presented in Figs. 1 and 2. The degradation of anthocyanins, phenolic acids and Q-3-G was carried out at 80, 90, 100, 110 and 120 °C, and mass fractions at all temperatures followed the first order reaction kinetics (Eq. 1) with a coefficient of determination R^2, ranging from 0.85 to 0.99 (Tables 1 and 2). These results are in accordance with previous reports (*36,38,40*). After 50 min of heating at 110 °C, Cy-3-G was the most unstable anthocyanin, and overall anthocyanins were significantly decreased, by approx. 40 %, between 20 and 30 min of heating. Total degradation of Cy-3-G was observed after 20 min of heating at 110 and 120 °C. Degradation was substantially slower at lower, and faster at higher heating temperature, which indicates that the duration and temperature of heating have a strong influence on the anthocyanin stability (*41*).

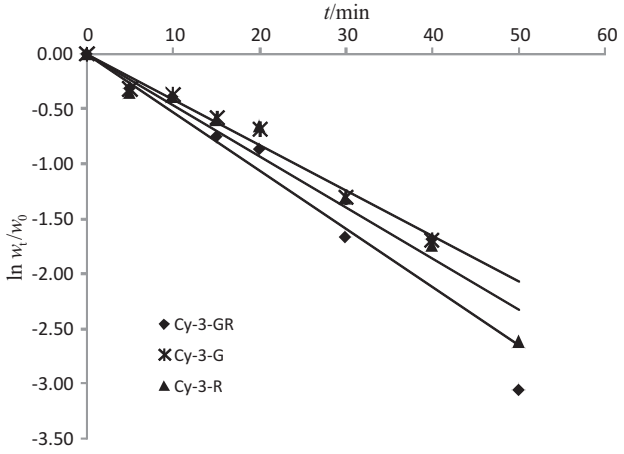

Fig. 1. Isothermal degradation of Cy-3-GR, Cy-3-G and Cy-3-R in freeze-dried sour cherry paste treated at 110 °C

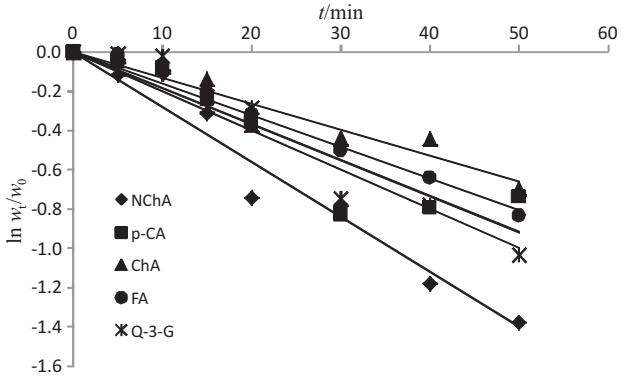

Fig. 2. Isothermal degradation of NChA, p-CA, ChA, FA and Q-3-G in freeze-dried sour cherry paste treated at 110 °C

The degradation of anthocyanins is primarily caused by oxidation, cleavage of covalent bonds or enhanced oxidation reactions due to thermal processing. Probably during heat treatment, anthocyanins or their conjugated sugars are broken down into small molecules such as aldehydes and benzoic acid derivatives or their corresponding anthocyanidins, respectively (*41,42*). Anthocyanin degradation is accelerated with increasing temperature, which supports the data of degradation rate constant *k* shown in Table 1.

Due to the above-mentioned reasons, the rate constant, *k*, as well as $t_{1/2}$ values at 100, 110 and 120 °C for Cy-3-G were not calculated. For the Cy-3-GR as a predominant anthocyanin in freeze-dried sour cherries, the degradation rate constant (*k*) increased from 2.2 to $8.5 \cdot 10^{-2}$ min^{-1} with the temperature increase from 80 to 120 °C. For the Cy-3-R, the degradation rate also showed a similar trend, with *k* increasing from 1.5 to $8.4 \cdot 10^{-2}$ min^{-1} as a result of temperature increase. Increasing the temperature for 40 °C (from 80 to 120 °C), *k* increased by approx. 4- to 5-fold. The activation energy (E_a) for the anthocyanin compounds in freeze-dried Marasca cherries was calculated according to the Arrhenius equation (Eq. 3). The resulting activation energies for anthocyanins (Table 1) were 42 kJ/mol for Cy-3-GR, 55 kJ/mol for Cy-3-G and 51 kJ/mol for Cy-3-R, with R^2 values ranging from 0.97 to 0.99. The obtained results are similar to the previously reported E_a for blackberry juice (58.95 kJ/mol) and raspberry juice (34.2 kJ/mol) (*36,43*). The E_a for anthocyanin degradation was higher in sour cherry concentrate than in juice; therefore, anthocyanins in the concentrate are more susceptible to thermal degradation (*34*).

The $t_{1/2}$ values of anthocyanins are expressed in Eq. 2 and presented in Table 1. The half-life at 110 °C of Cy--3-GR and Cy-3-R was 9.30 and 9.52 min, respectively. Cy-3-G was degraded after heating at temperatures higher than 90 °C. In this study, the half-life values of cyanidin glycosides in Marasca sour cherry paste ranged from 22.40 to 25.27 min at 90 °C. Cy-3-R was more susceptible to high temperatures than Cy-3-GR, while Cy-3-G was unstable. The half-life values of anthocyanin degradation in blackberry juice at 90 °C were from 3.85 to 4.97 h (*42*). Cemeroglu *et al*. (*34*) reported that $t_{1/2}$ value of anthocyanin degradation at 80 °C in sour cherry juice and concentrate was 8.1 and 2.8 h, respectively. These results for $t_{1/2}$ values of anthocyanin degradation were considerably higher, compared to the freeze-dried Marasca cherries analyzed in the present study. Another study (*44*) on pomegranate juices (clarified and cloudy) heated at 80 and 90 °C, reported higher $t_{1/2}$ values in clarified than in cloudy juices. This indicates that lower stability of anthocyanins in cloudy juices was due to the presence of macromolecular matrix compounds. The anthocyanin degradation was more rapid in more concentrated juices (*35*), probably due to increased solid content and reacting molecules (oxygen), which become closer and accelerate the rate of chemical reactions (*34,36*). Sugar and ascorbic acid present in fruits can also increase or decrease the anthocyanin degradation depending on their concentration (*45*). Different food matrix and chemical structure of anthocyanin-conjugated sugar (the type and place of

Table 1. Effect of temperature on the anthocyanin degradation in freeze-dried sour cherry Marasca paste

Compound	Temperature/°C	$k \cdot 10^{-2}$/min^{-1}	R^2	$t_{1/2}$/min^{-1}	E_a/(kJ/mol)	R^2
Cy-3-GR	80	2.2±0.2	0.87	32.10	42±2	0.98
	90	3.08±0.08	0.99	22.40		
	100	5.1±0.3	0.94	13.58		
	110	7.4±0.8	0.86	9.30		
	120	8.5±0.7	0.91	8.14		
Cy-3-G	80	1.6±0.1	0.94	41.81	55±2	0.99
	90	2.7±0.1	0.98	25.27		
	100	–		–		
	110	–		–		
	120	–		–		
Cy-3-R	80	1.5±0.1	0.91	45.69	51±2	0.97
	90	2.8±0.1	0.97	24.64		
	100	4.5±0.2	0.96	15.16		
	110	7.2±0.4	0.96	9.52		
	120	8.4±0.3	0.98	8.21		

Cy-3-GR=cyanidin-3-glucosylrutinoside, Cy-3-G=cyanidin-3-glucoside, Cy-3-R=cyanidin-3-rutinoside; k=degradation rate constant, $t_{1/2}$=half-life, E_a=activation energy

Table 2. Effect of temperature on the degradation of phenolic acids and flavonol glycosides in freeze-dried sour cherry Marasca paste

Compound	Temperature/°C	$k \cdot 10^{-2}$/min^{-1}	R^2	$t_{1/2}$/min^{-1}	E_a/(kJ/mol)	R^2
NChA	80	1.6±0.2	0.87	43.39	27±1	0.98
	90	1.9±0.2	0.90	36.13		
	100	2.8±0.1	0.96	24.64		
	110	3.2±0.2	0.93	21.90		
	120	3.9±0.2	0.95	17.60		
p-CA	80	1.12±0.07	0.95	61.60	23±1	0.98
	90	1.49±0.09	0.94	46.30		
	100	1.8±0.2	0.85	37.50		
	110	2.2±0.2	0.92	30.80		
	120	2.5±0.2	0.93	27.71		
ChA	80	1.0±0.1	0.85	66.99	8.1±0.9	0.78
	90	1.29±0.07	0.94	53.49		
	100	1.32±0.09	0.92	52.27		
	110	1.3±0.1	0.86	52.27		
	120	1.45±0.08	0.94	47.59		
FA	80	1.29±0.09	0.93	53.49	10.8±0.1	0.97
	90	1.50±0.07	0.96	46.00		
	100	1.61±0.06	0.98	42.85		
	110	1.70±0.09	0.95	40.58		
	120	1.9±0.2	0.92	35.94		
Q-3-G	80	1.5±0.1	0.90	46.94	18.1±0.2	0.94
	90	1.5±0.1	0.93	46.00		
	100	2.0±0.1	0.93	34.67		
	110	2.4±0.2	0.94	29.11		
	120	2.6±0.1	0.96	26.85		

NChA=neochlorogenic acid, *p*-CA=*p*-coumaric acid, ChA=chlorogenic acid, FA=ferulic acid, Q-3-G=quercetin-3-glucoside; k=degradation rate constant, $t_{1/2}$=half-life, E_a=activation energy

glycosylation, the presence of hydroxyl groups) probably affect its thermal stability (*46*). Consistently with previous reports, in freeze-dried Marasca cherries all compounds present in the matrix are concentrated, which may affect the anthocyanin stability.

The phenolic acids ChA, NChA, *p*-CA, FA, and Q-3-G are also among the most important BACs present in freeze-dried Marasca sour cheeries. Results show that the temperature and time of processing decreased the mass fractions of phenolic acids and Q-3-G, but they are more stable during heating compared to anthocyanins. The degradation of phenolic acids and Q-3-G in freeze-dried sour cherry paste samples is the same as of anthocyanins, but considerably slower (Fig. 2).

The most stable phenolic acids in freeze-dried Marasca cherry paste were ChA and FA because at the highest temperature they had the highest half-life. At 110 °C, $t_{1/2}$ values for ChA and FA were 52.27 and 40.58 min, respectively. Q-3-G had a lower $t_{1/2}$ value (29.11 min) than ChA and FA acids, but a considerably higher than cyanidin glycosides at the same temperature.

A considerable decrease in the mass fractions of ChA and FA occurred at a temperature of 110 °C after 20 and 30 min, respectively (Fig. 2). The same trend was observed for Q-3-G at the same temperature and time; its mass fraction decreased approx. 50 % after heating, respectively.

It is reported that the sugar moiety attached to the C-ring of flavone skeleton (3-O-position) is more susceptible to thermal degradation compared to other positions on it (*47*). According to van der Sluis *et al*. (*48*) various quercetin glycosides are the most thermally sensitive compounds, whereas chlorogenic acid is more stable in enriched apple juice.

The results show that the phenolic acids and Q-3-G in the freeze-dried Marasca cherry paste at higher temperatures (or during heat treatment) are more stable compared to anthocyanins. The reason for their greater stability compared to anthocyanins may be related to their chemical structure. The hydrolysis of sugar moiety at the position 3 of flavylium ring of anthocyanidin leads to a formation of colourless phenolic aglycones (chalcone), while the possible degradation mechanism is due to further transformations of aglycones into a coumarin glucoside derivative with a loss of the B-ring (*49*).

By increasing the heating temperature from 80 to 120 °C, the degradation rate constant (k) is increased by approx. 1.5- to 2.5-fold for phenolic acids and approx. 1.75-fold for Q-3-G. The degradation rate constant (k) of the ChA and FA, as the most stable phenolic acids in freeze-dried sour cherries, increased from 1.0 to $1.45 \cdot 10^{-2}$ min^{-1} and from 1.29 to $1.9 \cdot 10^{-2}$ min^{-1}, respectively. Degradation rate constant (k) for Q-3-G ranged from 1.5 to $2.6 \cdot 10^{-2}$ min^{-1} as a result of temperature increase. Increasing the heating temperature increased the k values, and hence accelerated the degradation rate.

At 120 °C, ChA and FA also had the highest half-life, 47.59 and 35.94 min, respectively. Q-3-G had a similar $t_{1/2}$ value to ChA and FA acids, respectively, and compared to anthocyanins was considerably more stable (Tables 1 and 2). Limited data concerning temperature sensitivity of individual nonanthocyanin polyphenolic compounds are available.

The activation energies (E_a) for phenolic acids and Q-3-G in freeze-dried Marasca cherries paste were as follows: ChA 8.1, FA 10.8, *p*-CA 23, NChA 27 and Q-3-G 18.1 kJ/mol, with R^2 values ranging from 0.78 to 0.98. Phenolic acids and Q-3-G had considerably lower E_a, but due to lower k values, these polyphenols were more stable to thermal degradation compared to anthocyanins.

The integrated models for temperature and time effects are presented as 3D surface plots in Figs. 3–5. They show that a series of isothermal experiments are well predicted by a single model (surface), which enables extrapolations of freeze-dried sour cherry Marasca prod-

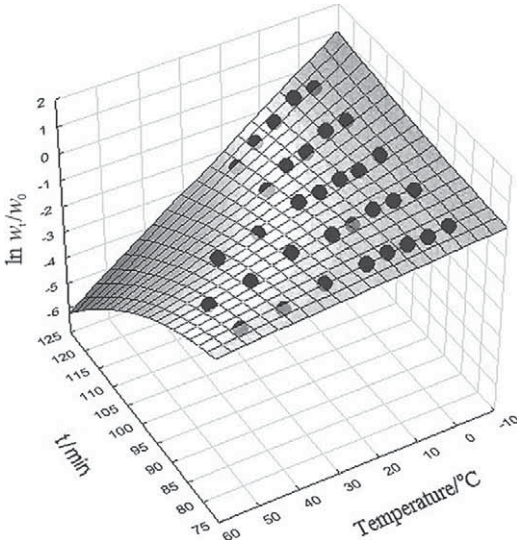

Fig. 3. 3D plot of the logarithm of mass fractions of Cy-3-GR as a function of temperature and time. The model is represented by the surface and each experimental data is presented by a black dot

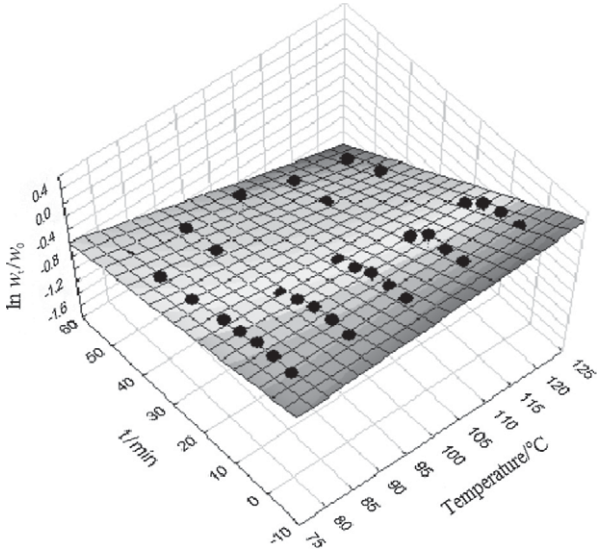

Fig. 4. 3D plot of the logarithm of mass fractions of *p*-CA as a function of temperature and time. The model is represented by the surface and each experimental data is presented by a black dot

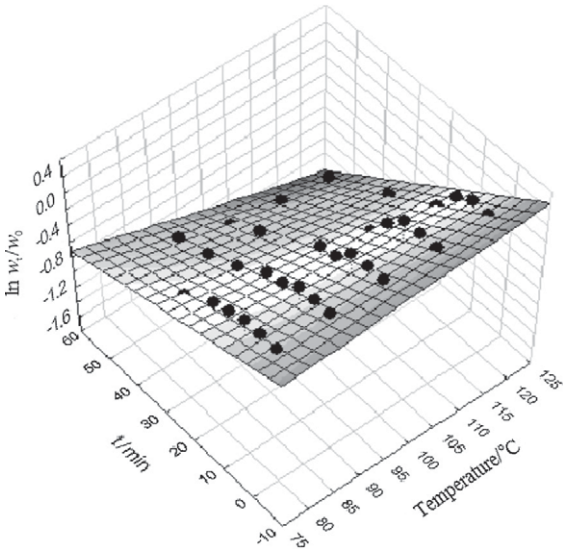

Fig. 5. 3D plot of the logarithm of mass fractions of Q-3-G as a function of temperature and time. The model is represented by the surface and each experimental data is presented by a black dot

ucts at lower temperatures (4 °C) at extended time. The results for Cy-3-GR, *p*-CA and Q-3-G are presented, with good precisions for reference rate constants and energies of activation. The precisions are expressed with p=0.0051 for the rate constant for Cy-3-GR, p=0.00015 for the rate constant of *p*-CA and p=0.00005 for Q-3-G. The p-values for all energies of activation are almost negligible. Precisions of the parameters of integrated models enable good predictions of the storage life of freeze-dried sour Marasca products.

Conclusion

Changes in kinetic stability of freeze-dried Marasca sour cherry paste during heating were evaluated. The results show that the degradation of anthocyanins, flavonol glycosides and phenolic acids of freeze-dried Marasca sour cherries follows the first order reaction kinetics and their degradation increases with the increase of temperature. The obtained energies of activation for Marasca anthocyanins and other polyphenolic compounds are in the same range as the data found in other fruits and products rich in anthocyanins.

The mass fractions of anthocyanins in freeze-dried Marasca cherries declined rapidly during 20 min of heating at 110 and 120 °C. Phenolic acids and flavonol glycosides are considerably more stable compounds and less susceptible to degradation at higher temperatures. Rapid anthocyanin degradation rate of freeze-dried Marasca sour cherries indicates that it is very important to identify suitable storage conditions, for which further studies are still needed. The applied integrated model for time and temperature effects on the concentrations of biologically active compounds derived from isothermal experiments shows its potential application for the estimation of changes during shelf-life under various conditions.

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References

1. J. Wollgast, E. Anklam, Review on polyphenols in *Theobroma cacao*: Changes in composition during manufacture of chocolate and methodology for identification and quantification, *Food Res. Int. 33* (2000) 423–447.
2. J.J. Macheix, A. Fleuriet, J. Billot: *Fruit Phenolics*, CRC Press, Inc., Boca Raton, FL, USA (1990).
3. G. Mazza, J.E. Cacace, C.D. Kay, Methods of analysis for anthocyanins in plants and biological fluids, *J. AOAC Int. 87* (2004) 129–145.
4. W. Zheng, S.Y. Wang, Antioxidant activity and phenolic compounds in selected herbs, *J. Agric. Food Chem. 49* (2001) 5165–5170.
5. B. Ou, K.N. Bosak, P.R. Brickner, D.G. Iezzoni, E.M. Seymour, Processed tart cherry products – Comparative phytochemical content, *in vitro* antioxidant capacity and *in vitro* anti-inflammatory activity, *J. Food Sci. 77* (2012) 105–112.
6. G.G. Duthie, S.J. Duthie, J.A.M. Kyle, Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants, *Nutr. Res. Rev. 13* (2000) 79–106.
7. A. Scalbert, C. Manach, C. Morand, C. Rémésy, L. Jiménez, Dietary polyphenols and the prevention of diseases, *Crit. Rev. Food Sci. Nutr. 45* (2005) 287–306.
8. J.K. Prasain, S.H. Carlson, J.M. Wyss, Flavonoids and age-related disease: Risk, benefits and critical windows, *Maturitas, 66* (2010) 163–171.
9. G. Mazza, E. Miniati: *Anthocyanins in Fruits, Vegetables and Grains*, CRC Press, Boca Raton, FL, USA (1993).
10. S. Pedisić, V. Dragović-Uzelac, B. Levaj, D. Škevin, Effect of maturity and geographical region on anthocyanin content of sour cherries (*Prunus cerasus* var. marasca), *Food Technol. Biotechnol. 48* (2010) 86–93.
11. A. Šarić, T. Balog, S. Sobočanec, B. Kušić, V. Šverko, G. Rusak *et al.*, Antioxidant effects of flavonoid from Croatian *Cystus incanus* L. rich bee pollen, *Food Chem. Toxicol. 47* (2009) 547–554.
12. S. Pedisić, B. Levaj, V. Dragović-Uzelac, K. Kos, Physicochemical composition, phenolic content and antioxidant activity of sour cherry cv. marasca during ripening, *Agric. Conspec. Sci. 72* (2007) 295–300.
13. L.G. Marques, A.M. Silveira, J.T. Freire, Freeze-drying characteristics of tropical fruits, *Drying Technol. 24* (2006) 457–463.
14. S. Grabowski, M. Marcotte, M. Poirier, T. Kudra, Drying characteristics of osmotically pretreated cranberries – Energy and quality aspects, *Drying Technol. 20* (2002) 1989–2004.
15. A.P.K. Joshi, H.P.V. Rupasunghe, S. Khanizadeh, Impact of drying processes on bioactive phenolics, vitamin C and antioxidant capacity of red-fleshed apple slices, *J. Food Process. Preserv. 35* (2011) 453–457.
16. H.H. Orak, T. Aktas, H. Yagar, S.S. Isbilir, N. Ekinci, F.H. Sahin, Effects of hot air and freeze drying methods on antioxidant activity, colour and some nutritional characteristics of strawberry tree (*Arbutus unedo* L) fruit, *Food Sci. Technol. Int. 18* (2012) 391–402.
17. J.W. Rhim, Kinetics of thermal degradation of anthocyanin pigment solutions driven from red flower cabbage, *Food Sci. Biotechnol. 11* (2002) 361–364.

18. G. Gradinaru, C.G. Biliaderis, S. Kallithraka, P. Kefalas, C. Garcia-Viguera, Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: Effects of copigmentation and glass transition, *Food Chem. 83* (2003) 423–436.

19. A. Hager, L.R. Howard, R.L. Prior, C. Brownmiller, Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed black raspberry products, *J. Food Sci. 73* (2008) 134–140.

20. A. Castañeda-Ovando, M.D.L. Pacheco-Hernández, M.E. Páez-Hernández, J.A. Rodríguez, C.A. Galán-Vidal, Chemical studies of anthocyanins, *Food Chem. 113* (2009) 859–871.

21. M. Alonzo-Macías, A. Cardador-Martínez, S. Mounir, G. Montejano-Gaitán, K. Allaf, Comparative study of the effects of drying methods on antioxidant activity of dried strawberry (*Fragaria* var. Camarosa), *J. Food Res. 2* (2013) 92–107.

22. L. Havlikova, K. Mikova, Heat stability of anthocyanins, *Z. Lebensm. Untersuch. Forsch. 181* (1985) 427–432.

23. M. Drdak, P. Daucik, Changes of elderberry (*Sambucus nigra*) pigments during the production of pigment concentrates, *Acta Aliment. 19* (1990) 3–7.

24. R.L. Jackman, J.L. Smith: Anthocyanins and Betalains. In: *Natural Food Colorants*, G.A.F. Hendry, J.D. Houghton (Eds.), Blackie Academic & Professional, London, UK (1996) pp. 280–309.

25. N. Asafi, B. Cemeroğlu, Degradation of anthocyanins in sour cherry and pomegranate juice and concentrates, *GIDA, 25* (2000) 407–411 (in Turkish).

26. M. Kopjar, M. Oršolić, V. Piližota, Anthocyanins, phenols and antioxidant activity of sour cherry puree extracts and their stability during storage, *Int. J. Food Prop.* (in press).

27. D. Fracassetti, C. Del Bo', P. Simonetti, C. Gardana, D. Klimis-Zacas, S. Ciappellano, Effect of time and storage temperature on anthocyanin decay and antioxidant activity in wild blueberry (*Vaccinium angustifolium*) powder, *J. Agric. Food Chem. 61* (2013) 2999–3005.

28. R.M. Syamaladevi, S.S. Sablani, J. Tang, J. Powers, B.G. Swanson, Stability of anthocyanins in frozen and freeze--dried raspberries during long-term storage: In relation to glass transition, *J. Food Sci. 76* (2011) 414–421.

29. I. Elez Garofulić, V. Dragović-Uzelac, A. Režek Jambrak, M. Jukić, The effect of microwave assisted extraction on the isolation of anthocyanins and phenolic acids from sour cherry Marasca (*Prunus cerasus* var. Marasca), *J. Food Eng. 117* (2013) 437–442.

30. M.N. Mitić, M.V. Obradović, D.A. Kostić, R.J. Micić, E.T. Pecev, Polyphenol content and antioxidant activity of sour cherries from Serbia, *Chem. Ind. Chem. Eng. Q. 18* (2012) 53–62.

31. F.A. Tomás-Barberán, M.I. Gil, P. Cremin, A.L. Waterhouse, B. Hess-Pierce, A.A. Kader, HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums, *J. Agric. Food Chem. 49* (2001) 4748–4760.

32. V. Šimunić, S. Kovač, D. Gašo-Sokač, W. Pfannhauser, M. Murković, Determination of anthocyanins in four Croatian cultivars of sour cherries (*Prunus cerasus*), *Eur. Food Res. Technol. 220* (2005) 575–578.

33. A. Kirakosyan, E.M. Seymour, D.E. Urcuyo Llanes, P.B. Kaufman, S.F. Bolling, Chemical profile and antioxidant capacities of tart cherry products, *Food Chem. 115* (2009) 20–25.

34. B. Cemeroglu, S. Velioglu, S. Isik, Degradation kinetics of anthocyanins in sour cherry juice and concentrate, *J. Food Sci. 59* (1994) 1216–1218.

35. G.A. Garzon, R.E. Wrolstad, Comparison of the stability of pelargonidin-based anthocyanins in strawberry juice and concentrate, *J. Food Sci. 67* (2002) 1288–1299.

36. W.D. Wang, S.Y. Xu, Degradation kinetics of anthocyanins in blackberry juice and concentrate, *J. Food Eng. 82* (2007) 271–275.

37. A. Patras, N.P. Brunton, C. O'Donnell, B.K. Tiwari, Effect of thermal processing on anthocyanin stability in foods; Mechanisms and kinetics of degradation, *Trends Food Sci. Techol. 21* (2010) 3–11.

38. L. Verbeyst, I. Oey, I. Van der Plancken, M. Hendrickx, A. Van Loey, Kinetic study on the thermal and pressure degradation of anthocyanins in strawberries, *Food Chem. 123* (2010) 269–274.

39. E. Piasecka, M. Uczciwek, R. Klewicki, D. Konopacka, M. Mieszczakowska-Frac, M. Szulc *et al.*, Effect of long-time storage on the content of polyphenols and ascorbic acid in osmo-convetively dried and osmo-freeze-dried fruits, *J. Food Process. Preserv.* (2012) 1745–4549.

40. N. Harbourne, J.C. Jacquier, D.J. Morgan, J.G. Lyng, Determination of the degradation kinetics of anthocyanins in a model juice system using isothermal and non-isothermal methods, *Food Chem. 111* (2008) 204–208.

41. E. Sadilova, F.C. Stintzing, R. Carle, Thermal degradation of acylated and nonacylated anthocyanins, *J. Food Sci. 71* (2006) 504–512.

42. L. Zhang, J. Zhou, H. Liu, M.A. Khan, K. Huang, Z. Gu, Compositions of anthocyanins in blackberry juice and their thermal degradation in relation to antioxidant activity, *Eur. Food Res. Technol. 235* (2012) 637–645.

43. M. Cissé, M. Dornier, M. Sakho, A. N'Diaye, M. Reynes, O. Sock, The roselle (*Hibiscus sabdariffa* L.): Composition and principal uses, *Fruits, 64* (2009) 179–193 (in French).

44. U.A. Fischer, R. Carle, D.R. Kammerer, Thermal stability of anthocyanins and colourless phenolics in pomegranate (*Punica granatum* L.) juices and model solutions, *Food Chem. 138* (2013) 1800–1809.

45. D. Svensson, Effects of heat treatment and additives on the anthocyanin content in blackcurrants and its relation to colour and texture, *MSc Thesis*, University of Technology, Göteborg, Sweden (2010) (in English).

46. J. Dai, A. Gupte, L. Gates, R.J. Mumper, A comprehensive study of anthocyanin-containing extracts from selected blackberry cultivars: Extraction methods, stability, anticancer properties and mechanisms, *Food Chem. Toxicol. 47* (2009) 837–847.

47. S. Rohn, N. Buchner, G. Driemel, M. Rauser, L.W. Kroh, Thermal degradation of onion quercetin glucosides under roasting conditions, *J. Agric. Food Chem. 55* (2007) 1568–1573.

48. A.A. van der Sluis, M. Dekker, M.A. van Boekel, Activity and concentration of polyphenolic antioxidants in apple juice. 3. Stability during storage, *J. Agric. Food Chem. 53* (2005) 1073–1080.

49. U. Tiwari, E. Cummins, Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations, *Food Res. Int. 50* (2011) 497–506.