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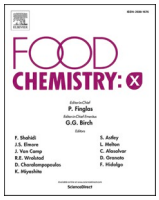

# Estimation of degradation kinetics of bioactive compounds in several lingonberry jams as affected by different sweeteners and storage conditions

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ABSTRACT

Lingonberry fruits are considered to play an important role in nutrition, as they comprise a variety of health-promoting components. Because of lingonberries seasonal availability and also due to their rapid degradation, their stability during processing is a continuous challenge for the food industry. Lingonberries are ideal fruits in making jam due to their natural deep reddish color, but recently, increased demand for low-calorie jams with alternative sweeteners has gained special attention. In this line, the objective of this study was to monitor the changes in anthocyanins, vitamin C, total phenolics, total reducing sugars and antioxidant capacity of several lingonberry jams formulated with different sweeteners (sucrose, fructose, erythritol, brown sugar, coconut sugar, stevia, saccharin). Due to the fact that storage conditions are important factors for jam quality, the jams were stored for 180 days at 4 °C and 25 °C (both under light and dark conditions). The rate constants (k) and the half time values ($t_{1/2}$) of the degradation processes were determined and degradation kinetics was studied. For all analyzed conditions, first-order reaction kinetics was established for the degradation process of anthocyanins, whereas a second-order kinetic model described the degradation of the other compounds. Kinetic parameters showed that the stability of the studied compounds was highly influenced by the type of sweetener used in jam formulation. Total phenolics and antioxidants were best preserved in the presence of stevia, coconut sugar and fructose, whereas a destabilizing effect of erythritol on vitamin C and anthocyanins content during storage was observed. Among all the studied compounds, anthocyanins presented the fastest degradation, regardless storage conditions. The stability of studied compounds was higher at lower storage temperature (4 °C), while increasing the temperature at 25 °C and exposure to light determined higher rate of the degradation processes. The results provide useful information for understanding some bioactive compounds degradation in real foods, contributing to the development of new food products and providing information of commercial importance.

## Introduction

Lingonberry (*Vaccinium vitis-idaea* L.) is widely consumed both as fresh fruit and processed products such as lingonberry jam or others. It is a very rich source of anthocyanins, proanthocyanidins, flavonols and phenolic acids (Brown et al., 2016). In addition to these nutrients, lingonberries also contain vitamins (C, B1, B2, B3 and A), potassium, calcium, magnesium and phosphorous (Dróżdż et al., 2018). Phenolics are responsible for the wide range of biological properties of these fruits, among which the antimicrobial, anticarcinogenic and antiproliferative activity (Brown et al., 2016; Zheng & Wang, 2003; Fan et al., 2011). Moreover, these fruits are a valuable source of natural sugars (fructose, glucose and low amounts of sucrose), responsible to have a major effect on taste, fruit ripeness, or even present an index of consumer acceptability (Mikulic-Petkovsek et al., 2012; Vilkickyte et al., 2019).Sugar amounts in lingonberries are not dangerously high, and consequently, do not seem to be hazardous for consumers (Vilkickyte et al., 2019). Due to these properties and to the fact that lingonberries are seasonal fruits, different ways of preserving them are applied, for example in the form of jams, as a good alternative for consumers.

Jam is a stable product made from fruits and sugar generally combined in similar ratios. However, high sugar intake can cause metabolic disease such as obesity and diabetes (Ji Yeon et al., 2016). On the other hand, nowadays, consumers are looking for natural products with

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particular nutritive value. Therefore, it is important to replace sugar by other substances which maintain the sweet taste of the product in order to improve health functions in the population. The coconut and brown sugars would be two sugar options that are preferred instead of the traditional white sugar. Brown sugar does not undergo refinement steps and becomes a valuable nutritional product from the sugarcane industry, while coconut sugar is considered one of the healthiest sugars due to the nutrients contained (Curi et al., 2017). Stevia has also recently gained importance as natural non caloric sweetener that contains essential compounds and has not been reported as being hazardous to health, such as saccharin (Gasmalla et al, 2014). Fructose is also often recommended due to its very low glycemic index (GI) (Belovićet al., 2017), especially for diabetic products.

Processing and storage conditions may strongly affect jam quality parameters, such as antioxidant properties, phenolics, anthocyanins content, as well as sensory properties (Belović et al., 2017; Benedek et al., 2020; Scrob et al., 2021). The sweeteners (natural or synthetic) may also affect the stability of these compounds, so the type of sweetener added to the jams should be carefully selected in order to minimize the degradation of final product during storage (Moldovan & David, 2020). For example, using fructose as a sweetener may negatively affect the stability of black currants anthocyanins, while aspartame and sucrose may enhance their stability (Rubinskiene et al., 2005). Therefore, investigating the effect of different sweeteners on the stability of bioactive compounds from various fruit products may play a key role for food industry development.

Since lingonberry fruits are not available all the year and the jam industry needs to improve its competitiveness and develop new products, the research hypothesis is that the sweeteners used in jams formulations have an effect on stability of bioactive compounds during storage, this being a critical aspect in terms of evaluating the real potential of any jams. In this context, the objective of this study was to estimate the influence of different types of sweeteners on the stability of total phenolics content (TPC), total anthocyanins content (TAC), vitamin C, reducing sugars and antioxidant capacity (AC) of lingonberry jams during different storage conditions. To the best of our knowledge, this is the first study on the impact of sweeteners on the degradation of bioactive compounds in lingonberry jams. Kinetic data regarding the degradation of these compounds is essential to predict quality losses during storage and will facilitate the development of new food products.

## Materials and methods

### *Reagents and chemicals*

All chemical reagents used in this study were of analytical grade. Ethanol 96 %, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), $K_2S_2O_8$, Folin–Ciocalteu reagent, ascorbic acid, 3,5-dinitrosalicylic acid (DNS), sodium potassium tartrate, NaOH, $Na_2CO_3$, KCl, $CH_3COONa$, $KMnO_4$, 36.5 % HCl, glacial acetic acid, $H_2SO_4$ (0,1N) were purchased from Merck (Darmstadt, Germany). Ultrapure water was used in all the experiments. Sucrose was purchased from a local market in Cluj-Napoca, while all the other sweeteners used in this study were purchased from a health food store.

### *Fruits material and sample preparation*

Fresh lingonberries (*Vaccinium vitis-idaea* L.) were harvested from Apuseni Mountains, Romania, in August 2019, where no agronomic practices have been done during growing. They were sorted, washed immediately after harvest, and inedible parts were rejected. Fruits were frozen as a whole, and kept in polypropylene bags until preparation of jams.

Lingonberry jams were prepared in the laboratory, according to a traditional procedure, with no thickening agents, stabilizers or other preservatives added, by boiling in an open kettle, with manual stirring. Fruits were ground prior to jam preparation with a mixer (Philips Daily Collection HR7510/00, 800 W). All jams were prepared using 100 g of fruit and the following quantities of sweeteners:50 g sucrose (Jam 1), 29.4 g fructose (Jam 2), 77.0 g erythritol (Jam 3), 50.0 g brown sugar (Jam 4), 50.0 g coconut sugar (Jam 5), 0.180 g stevia (Jam 6) and 0.180 g saccharin (Jam 7). Different quantities of sweeteners used for jam preparation were determined in order to keep the same sweetness index as Jam 1 (control). The ingredients were heated at low temperature (50 °C) to prevent the degradation of compounds. Heating process was stopped when total soluble content (TSS) reached value of 56-57° Brix (Kamiloglu et al., 2015), excepting Jam 6 and Jam 7, formulated with stevia and saccharin, respectively. In the case of the last two jams, heating was stopped when TSS reached about 22°Brix, as previously reported by Sutwal et al. (2019). Each jam was packed into glass jars with screw caps, without being pasteurized. After the jams were cooled to room temperature, each type of jam was divided into three groups. The first group was stored in the refrigerator at 4 °C, the second under light at 25°Cand the third under dark at 25 °C. Samples were analyzed immediately and after 15, 30, 60 and 180 days of storage.

The jam extracts were prepared as following: 1.0 g of lingonberry jam accurately weighted was mixed with 4.5 mL ethanol and 5.5 mL ultrapure water and stirred for 1 h at room temperature on the magnetic stirrer. Extraction was then performed at 30 °C for 30 min in an ultrasonic thermostatic bath Elmasonic E60H (Elma Schmidbauer GmbH, Singen am Hohentwiel, Germany). The extracts were centrifuged at 875g for 20 min using a Centurion Scientific centrifuge C2006 (Centurion Scientific Limited, Bosham, UK). Each extract was filtered out using filter paper and the supernatants were collected and used for further analyses.

### *Spectrophotometric assays*

A T80 + UV–vis spectrophotometer (PG Instruments, Lutterworth, UK) was used for spectrophotometric measurements. In all cases the samples were analyzed in triplicate.

### *Determination of vitamin C*

The quantification of vitamin C was performed by a spectrophotometric method using potassium permanganate (Zanini et al., 2018). Concisely, aliquots of 2.0 mL of each extract optimally diluted were mixed with 2.0 mL of 0.1 mol/L $KMnO_4$ prepared in $H_2SO_4$. Immediately after mixing, the absorbance of the samples was read at 525 nm. The blank was measured in the same way replacing the sample with ultrapure water. The decrease of the absorbance (ΔA) was calculated using the equation (1):

$$\Delta A = A_{blank} - A_{sample} \quad (1)$$

The content of vitamin C was obtained from the calibration curve achieved in the same conditions and was expressed as mg/g jam.

### *Total anthocyanins content (TAC)*

TAC in the extracts was determined by pH-differential method (Moldovan et al., 2015). Ethanolic extracts of jams (1 mL) were diluted with 3 mL of KCl/HCl buffer, 0.025 M, pH = 1 and $CH_3COONa$/$CH_3COOH$ buffer, 0.4 M, pH = 4.5. After 15 min, the absorbance of each solution was measured at $\lambda^{max}$ – 532 nm and at 700 nm. TAC was calculated using equation (2) being expressed as cyanidin-3-glucoside equivalents (mg Cy-3-gly/L):

$$TAC = (A \times MW \times DF \times 1000)\ (\varepsilon \times l) \quad (2)$$

MW = molecular weight (449.2 g/mol); DF = dilution factor; l = path length (1 cm); ε = molar extinction coefficient (26900 L/mol·cm); 1000 = conversion factor from gram to milligram. Absorbance (A) was calculated by equation (3):

$$A = (A_{pH\ 1.0} - A_{pH\ 4.5})_{532\ nm} - (A_{pH\ 1.0} - A_{pH\ 4.5})_{700\ nm} \quad (3)$$

Finally, the TAC was expressed as cyanidin-3-glycoside equivalents (mg Cy-3-gly/g jam).

*Total phenolics content (TPC)*

TPC was determined using the Folin-Ciocalteu reagent as described by Singleton et al. (1999), with some modification. Briefly, 0.3 mL of extract (properly diluted with ultrapure water) was mixed with 1.5 mL Folin-Ciocalteu's reagent (0.2 N), followed by addition of 1.2 mL sodium carbonate (0.7 M). The absorbance of the mixture was measured at 760 nm after incubation at room temperature for 2 h. The results were expressed as mg of gallic acid (GAE)/g jam.

*Antioxidant capacity (AC) measurement*

Discoloration of ABTS•$^+$ solution was determined according to a slightly modified method of Re et al. (1999). ABTS•$^+$ was generated by mixing equal volumes of ABTS solution (7 mM) and $K_2S_2O_8$ solution (2.45 mM) and kept in the dark at room temperature for 24 h. The ABTS•$^+$ was diluted before use so that its absorbance was around 0.800. A volume of 3 mL ABTS•$^+$diluted solution was mixed with 0.5 mL properly diluted extract and the absorbance of the mixture was measured at 734 nm after 15 min. The results were expressed in terms of Trolox equivalents (μmols/g jam) based on the calibration curve.

*Determination of reducing sugars*

Total reducing sugars content was determined using the DNS method (Garriga et al., 2017), which consists of a redox reaction between the 3,5-dinitrosalicyclic acid and the reducing sugars present in the sample. DNS reagent was prepared by mixing the solution A (1.00 g of DNS dissolved in 20 mL of NaOH 2 M) with solution B (30 g of sodium and potassium tartrate tetrahydrate dissolved in 50 mL of distilled water) under heat until homogenization. The volume was completed to 100 mL with distilled water and the reagent was stored in amber bottle at 4 °C. In the tubes of 10 mL, the properly diluted sample (1 mL) and DNS reagent (1 mL) were mixed together. The tubes were placed in a thermostated bath at 100 °C for 5 min and then cooled to room temperature. The volume was completed with 8 mL of distilled water, homogenized and the absorbance of the sample was read at 540 nm. The blank was obtained in the same way using ultrapure water instead of sample. The results were obtained from the calibration curve of D-glucose and were expressed as mg/g jam.

*Degradation kinetics*

The vitamin C, TAC, TPC, AC and total reducing sugars change during storage were described by fitting the experimental data with the zero-order (Eq. (4)), first-order (Eq. (5)) or second-order (Eq. (6)) kinetic model.

$$C = C_0 - kt;\ t_{1/2} = C_0/2k \quad (4)$$

$$C = C_0 \exp(-kt);\ t_{1/2} = \ln 2/k \quad (5)$$

$$1/C - 1/C_0 = k_{obs}t;\ t_{1/2} = 1/C_0k \quad (6)$$

where the $C_0$ and C is the value of parameter at initial time and after t time, $k$ is the reaction rate constant and $t_{1/2}$ is half-life period.

The reaction order was establish using a trial-and-error procedure, which supposes that if the order is assumed correctly the plot of the concentration *vs* time in the case of zero-order, ln concentration *vs* time in the case of first-order, and 1/concentration *vs* time in the case of second-order should be linear (Moratalla-López et al., 2019). The reaction order was selected for which the plot has the highest determination coefficient ($R^2$).

*Statistical analysis*

All experiments were conducted in triplicate and the obtained data were reported as mean ± standard deviation. T-test was used to determine significant differences between values. The significance level was defined as $p < 0.05$ for 95 % probability. To identify specific patterns in degradation rate of the determined parameters the factor analysis (FA) method was applied considering the variation of the analyzed parameters during the investigated period of 180 days of storage and the kinetic degradation parameters. Also, factorial ANOVA was applied for testing if some interaction between sweetener type and storage in order to know which factor acted as the source of the detected dissimilarity. Statistics 8.0 software package (StatSoftinc. 1984–2007, USA) was used for statistical analysis.

## Results and discussion

*Effects of sweeteners on main bioactive compounds of jam during different storage conditions*

*Vitamin C*

Nutritional properties of lingonberry fruits are also related to the content of vitamin C, which possesses a great antioxidant power, acts as an anti-scurvy disease, eliminates free radicals and minimizes damage to lipids, proteins and nucleic acids (Zanini et al., 2018). However, ascorbic acid is the most difficult vitamin to be preserved during storage due to its facility of degradation, especially at high temperatures and light (Sutwal et al., 2019). First the vitamin C is oxidized at dehydroascorbic acid, which is then degraded by hydrolysis to 2,3-diketogulonic acid. In order to get the maximum benefit of the vitamin C in lingonberry jams, manufacturers have to properly process and store the jams under appropriate conditions. Fig. 1a shows a gradual decrease of vitamin C content during 180 days of storage under different conditions. A significant decrease (Table 1) it can be observed after only 15 days of storage under light conditions in the case of all jam samples, indicating that vitamin C is rapidly degraded when exposed to light. As it can be seen from Fig. 1a, vitamin C is more stable at 4 °C in jams with stevia and coconut sugar, respectively, with losses ranging from 23.9 % in jam with stevia to 32.2 % in the case of jam formulated with coconut sugar. This suggests a stabilizing effect of stevia sweetener upon vitamin C content, which has been found by other authors (Kroyer, 2010). The vitamin C loss is also decreased in the case of jams stored under refrigeration. Burdurlu et al. (2006) confirmed in their previous study that lower temperature storage could prevent the degradation of vitamin C. The fact that vitamin C is better preserved at low temperatures is not surprising because it has been shown that its degradation reactions are accelerated with increasing temperature (Gregory, 2008).

*Total anthocyanins content (TAC)*

The wide spectrum of beneficial biological properties of lingoberries is also related to TAC, considered important quality indicators with potential antioxidant effects. However, anthocyanins are susceptible to light, high temperatures, ascorbic acid, etc (Hou et al., 2013). Anthocyanins degradation is reported by many authors in berry products and is partly attributed to indirect oxidation reactions. Losses of monomeric anthocyanins in berry preserves take place due to anthocyanin degradation and polymerization, during storage (Benedek et al., 2020). The degradation of TAC in lingonberry jams was monitored during 180 days of storage under different conditions and its variation can be seen in Fig. 1b. The *t*-test (Table 1) reveals that all jam samples exhibit a significant decrease in TAC after 15 days under light conditions, indicating the susceptibility of anthocyanins to light (Hou et al., 2013; Laleh et al., 2006). Even in dark conditions of storage, TAC is significantly lower after 30 days. A notable degradation of TAC, by approximately 99.9 % it can be observed in the case of jam with coconut sugar (Jam 5) during storage under dark. When stored at 4 °C, lingonberry jams have significant losses of TAC only after 60 days. Under refrigerator conditions, fructose show a good preservation effect for anthocyanins (Jam 2 with a loss of 36.2 %), while the use of erythritol lead to maximum loss of these

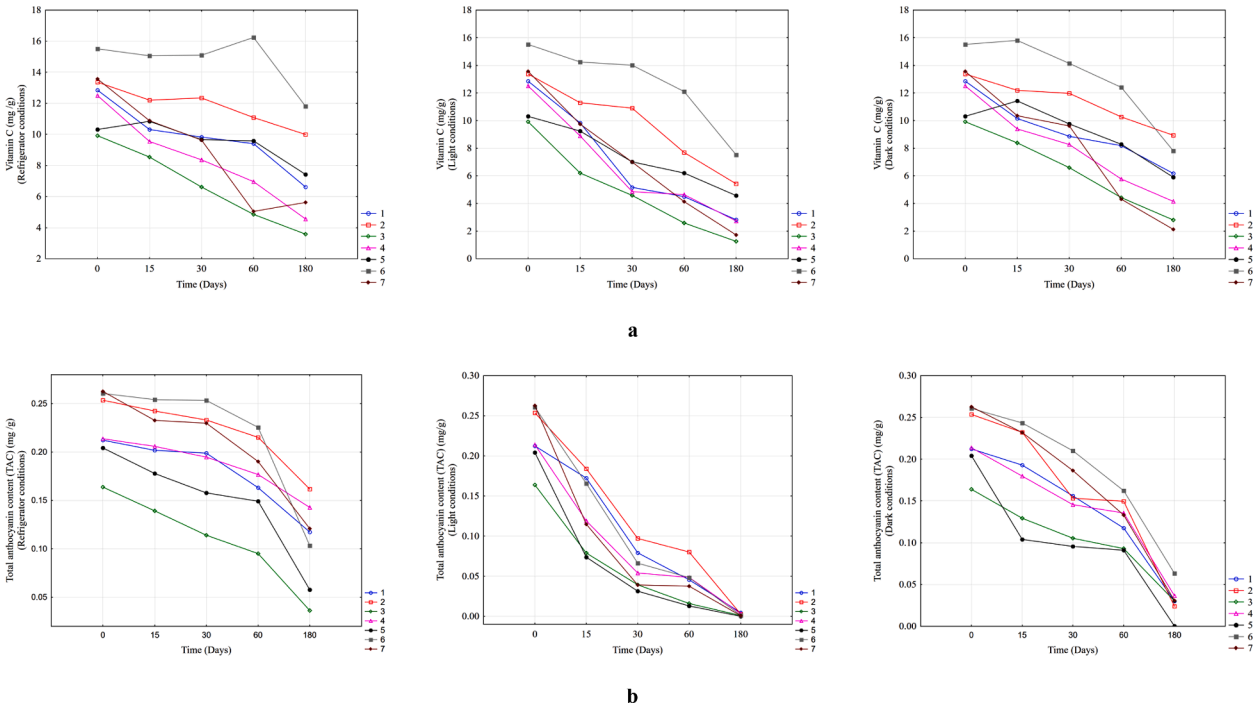


**Fig. 1.** Changes observed during 180 days under different storage conditions (refrigerator, light and dark) in Vitamin C (a), 1-sucrose; 2-fructose, 3-erythritolTAC (b), TPC (c), AC (d) and reducing sugars (e) in jams samples formulated with:; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharin.

**Table 1**
The Student's *t*-test results (*t*-values) for statistical comparison of determined parameters (TPC, TAC, vitamin C, AC and reducing sugars content) after different periods of storage under refrigerator, light and dark conditions respectively.

| Storage period(days) | Parameters / storage conditions (R, L, D) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TPC | | | TAC | | | Vitamin C | | | AC | | | Reducing sugars | | |
| | R | L | D | R | L | D | R | L | D | R | L | D | R | L | D |
| 15 | 0.35 | 0.76 | 0.51 | 0.82 | 4.34** | 1.50 | 1.41 | 2.25* | 1.26 | 0.75 | 1.36 | 0.86 | −0.44 | −0.99 | 15 |
| 30 | 0.65 | 1.58 | 0.87 | 1.18 | 10.09*** | 3.59* | 1.85 | 3.23* | 2.26* | 2.04 | 4.52** | 2.57* | −1.00 | −1.63 | 30 |
| 60 | 0.94 | 2.76* | 1.69 | 2.35* | 11.29*** | 5.74*** | 2.13 | 4.73** | 3.61* | 2.71* | 5.28** | 3.24* | −1.48 | −2.12 | 60 |
| 180 | 1.71 | 3.83* | 2.54* | 5.45** | 16.15*** | 12.53*** | 4.13** | 7.93*** | 5.96*** | 4.98** | 8.34*** | 5.95*** | −2.03 | −3.25* | 180 |

R – refrigerator storage conditions; L – light storage conditions; D – dark storage conditions.
Asterisks signify the levels of statistical significance of differences compared to initial values: * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

compounds (Jam 3 with a loss of 78.0 %).

*Total phenolics content*

Lingonberry consumption is also recommended due to their TPC that is reported to have a major contribution to the human health benefits. For extending shelf life and obtaining high-quality products, knowledge on the impact of processing and storage on these bioactive compounds should be very well documented (Brown et al., 2016; Stănciuc & Râpeanu, 2019). The changes in TPC as a function of storage time are presented in Fig. 1c. The storage at 25 °C results in a decrease of TPC, especially in the case of the light stored jams, whereas storage at 4 °C has no significant effect on TPC (Table 1). Following 180 days of storage under light conditions, lingonberry jams show a decline in TPC ranging from 48.6 % in the case of jam with fructose and 75.5 % in the case of jam with stevia, respectively. For dark stored jams at 25 °C, there are no significant losses of TPC in the first 60 days of storage, but there can be noticed a significant decrease in TPC after 180 days of storage, suggesting the influence of storage period upon phenolics stability. Coconut sugar is the sweetener that mainly protected TPC losses when jams were stored under dark, whereas in the case of erythritol jam it can be observed an opposite effect. Interestingly, when stored under refrigeration, erythritol shows a better protection upon TPC (22.6 % decrease), followed by fructose (23.3 % decrease) and coconut sugar (25.7 % decrease). It is important to note that storage at 4 °C gives significantly higher conservation of TPC compared with storage at 25 °C. The decrease of the TPC at higher temperatures may be due to increased oxidation of these bioactive components.

*Antioxidant capacity (AC)*

Degradation of anthocyanins, phenolics and other bioactive compounds may significantly influence the AC. Since a part of antioxidants present in lingonberry jams is lost during digestion (Scrob et al, 2022), it is of significant importance to develop products that maintain their antioxidant content during storage. Changes of the AC of lingonberry jams during storage are presented in Fig. 1d. As it can be seen, regardless of storage conditions, there is a decreasing tendency in the case of all jams. Light stored jams show the most pronounced decline in AC during the storage. In the case of jam with stevia, approximately 97.9 % of the original capacity is lost during 180 days of storage under light. When stored at 4 °C for 60 days, there are not significant decreases in AC of lingonberry jams (Table 1), but significant losses in AC were observed when storage was extended to 180 days. This indicates that prolonged storage may affect native antioxidants stability. A lower decrease of AC under refrigerated conditions is observed in the case of fructose jam

(Jam 2–30.5 %) and stevia jam (Jam 6–50.5 %). The quite high stability of antioxidants in stevia based jam is in accordance with previous studies (Pérez-Ramírez et al., 2015), where stevia incorporation in a roselle (*Hibiscus sabdariffa L.*) beverage lead to an increase in AC. The protective effect of steviol glycoside upon AC was also observed in a study on sour cherry puree (Nowicka & Wojdyło, 2016).

*Reducing sugars*

Sugars are important constituents of fruit products that act as natural food preservatives and influence the flavor of the food products (Sutwal et al., 2019). Also, the level of reducing sugars in fruits products are indicative of the quality of these food, and monitoring the levels of reducing sugars during food storage has improved market quality. For reducing sugars a gradual increasing is observed during the investigated period (Fig. 1e). The initial reducing sugars content is between 95.1 mg/g (Jam 3) and 415 mg/g (Jam2), but this high content of Jam 2 could be attributed to fructose, a reducing sugar, that is used as sweetener in jam formulation. The highest increase in reducing sugars content (4.50-fold) it can be observed in jam with sucrose (Jam 1) at the end of 180 days of storage under light conditions. The increase in reducing sugars might be due to the inversion of sucrose to reducing sugar and to hydrolysis of polysaccharide into simple sugar in the acidic environment during storage (Sutwal et al., 2019). It is important to note that reducing sugars content in light-stored jams was higher than those stored under dark conditions. Also, jams stored at 25 °C present higher reducing sugars content than jams stored at 4 °C, indicating that temperature and exposure to light are factors that mainly influence the concentration of these compounds during storage. Similar results in increasing of reducing sugar content have been reported by Sutwal et al. (2019) for low calorie apple jam by using stevia as a sweetener, Rana et al. (2021) for mixed fruit jam. Another explanation for the increase in reducing sugar content is the possible hydrolysis of phenolics in an acidic environment. The statistical analysis showed a negative and significant ($p < 0.05$) correlation between TPC and reducing sugar content for all jams (Pearson's correlation coefficient between 0.89 and 0.97), which supports the role of reducing sugars in the synthesis of various phenolics in lingonberry.

*Kinetic analysis*

*Vitamin C*

The loss of vitamin C in lingonberry jams during storage show better fits in a second-order kinetic model (Fig. 2) than in zero-order or first-order, which is in accordance with other studies (Robertson & Samaniego, 1986). Table 2 shows the kinetic parameters determined for vitamin C degradation during storage. The degradation rate of vitamin C has been found to be highest in erythritol jam (Jam 3) and the lowest in stevia jam (Jam 6) at both storage temperatures, proving the stabilizing effect of stevia sweetener and the accelerating degradation process in the presence of erythritol. This finding may suggest that adding stevia to lingonberry jams as a sweetener agent may contribute to vitamin C preservation during storage. This can be due to the protective effect of stevioside on the degradation of ascorbic acid, as reported by Kroyer (2010). Furthermore, coconut sugar addition reveals a good anti-degradation effect on vitamin C. In the case of jam formulated with coconut sugar stored under refrigeration, vitamin C has a half-life value more than 5 fold higher than jam with erythritol. This suggests that the degradation rate of vitamin C in lingonberry jams could be retarded by the addition of different sweetening agents. The low $t_{1/2}$values of lingonberry jams stored at 25 °C suggest that temperature and light exposure are factors that determine a faster degradation rate of vitamin C. This indicates the dependence of vitamin C degradation rate on storage temperature, being in agreement with other studies showing similar behavior (Martinsen, et al., 2020).

*Total anthocyanins content (TAC)*

During storage, regardless of the added sweetener, temperature or exposure to light, degradation of anthocyanins from lingonberry jams fits a first-order model with high determination coefficients ($R^2$ greater than 0.9668) (Fig. 2). This finding is in agreement with previous studies (Hou et al., 2013; Benedek et al, 2020; Moldovan & David, 2020). The use of different sweeteners in formulation of jams obviously affected the rates of these pigments degradation (Table 2). In jams sweetened with brown sugar and sucrose, respectively, TAC had the highest stability during storage under refrigeration. This might be due to a stabilizing effect of sugar on monomeric anthocyanins (Benedek et al., 2020). When erythritol is used in jam's formulation, the degradation at 4 °C occurred approximately 3.5-fold faster, indicating the weak effect of this sweetener on the stability of these components. Thus, sugar alcohols such as erythritol do not seem to be suitable choices in terms of preservation of lingonberry anthocyanins. Non-caloric sweeteners, such as stevia and saccharin, have generally shown slightly better TAC protection than erythritol. Light exposure of jam formulated with coconut sugar resulted in the highest increase of the k value, indicating a rapidly degradation of anthocyanins in the presence of this sweetener. This might be explained by the fact that different sugars and their degradation products tend to accelerate the degradation of anthocyanins. The rate of anthocyanins degradation is associated with the rate at which the sugar is degraded to furfural-type compounds (Hou et al., 2013).

Temperature has a significant role in destabilizing the anthocyanin molecular structure. As expected, the degradation rate of anthocyanins increases with the increase of temperature. By comparing the half-life values (Table 2), it can be observed that at 25 °C, lingonberry anthocyanins are more susceptible to degradation than they are at 4 °C, regardless of sweetener agent. For the samples stored at 25 °C both under light and dark conditions, coconut sugar determines the lowest stability of anthocyanin pigments, followed by fructose. Moldovan et al. (2020) also reported a negative effect of fructose on the anthocyanin stability. This can be attributed to the enhanced rate of the Maillard reaction in the presence of fructose and thus to the formation of furfural derivates that have a destructive effect on anthocyanins, as mentioned earlier (Benedek et al., 2020). Light is another factor that affects the stability of anthocyanins in the case of all jams, leading to a significant loss of color during storage. When exposed to light and temperature, the degradation rate of brown sugar jam increases almost 10-fold (Table 2). The effect of light on accelerating the degradation of anthocyanins in four *Berberis* species has been studied by Laleh et al.(2006), proving more than 85 % of TAC were degraded after keeping the samples in the light for 84 days at 25 °C. Thus, low temperature storage and absence of light may be preferred for high anthocyanins stability in lingonberry jams.

*Total phenolics content (TPC)*

The analysis of the data shows that the second-order model best fits the experimental data in the case of TPC, with highest $R^2$ values ranging from 0.8999 to 0.9882 (Fig. 2) even if many studies found that phenolics do not follow a specific reaction order (Benedek et al., 2020). Temperature and exposure to light are the factors that most affected the phenolics degradation (Table 2). The k values show strong temperature dependence, with higher values measured at 25 °C than at 4 °C. For example, in the case of erythritol jam, the degradation rate of phenolics stored under dark at 25 °C is 3.28 faster as compared to degradation at 4 °C while, under light conditions is almost 8 times faster. The results indicate that a higher storage temperature causes a faster decrease in TPC, being consistent with results from previous studies on phenolics degradation from blueberry jam (Scibisz and Mitek, 2009).

The presence of different sweeteners affects the degradation rate of TPC during storage. The jam formulated with sucrose presented a degradation rate of $0.870 \cdot 10^{-3}$ $day^{-1}$ under refrigeration, which was decreased 4.14 times by the substitution of sucrose with coconut sugar and 2.28 times in the case of stevia based jam. A similar trend has been

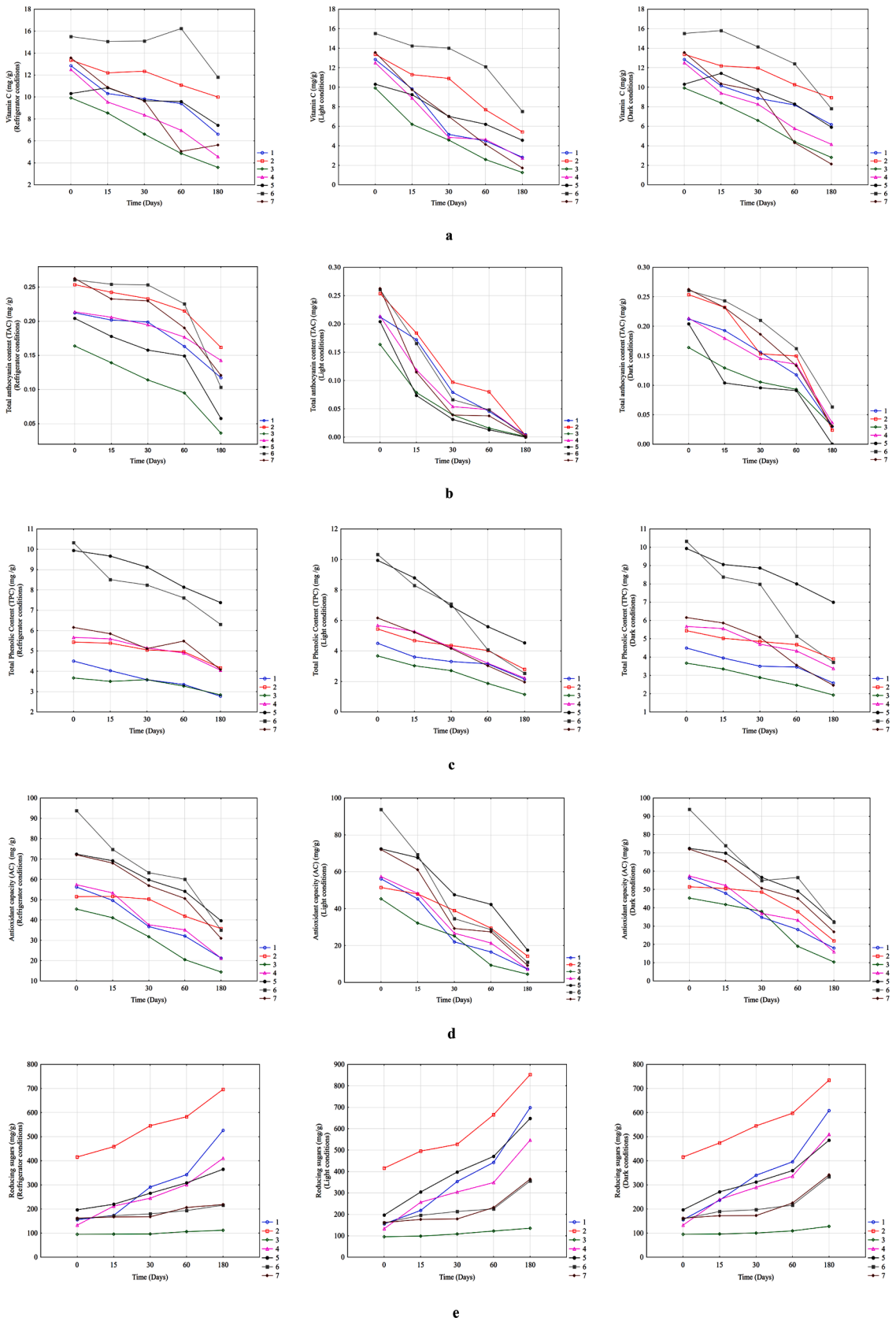


**Fig. 2.** Degradation of the Vitamin C, TAC, TPC, AC and accumulation of reducing sugars in jam samples under different storage conditions.

**Table 2**
Kinetic parameters for the degradation process of Vitamin C, TAC, TPC and AC, and for the accumulation process of reducing sugars from lingonberry jams after different periods of storage under refrigerator (R), light (L) and dark (D) conditions respectively. Rate constants are expressed as $k \cdot 10^{-3}$ ($day^{-1}$) and half-life values ($t_{1/2}$) are expressed in days.

| Sample | Vitamin C | | | | | | TAC | | | | | | TPC | | | | | | AC | | | | | | Reducing sugars | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | | L | | D | | R | | L | | D | | R | | L | | D | | R | | L | | D | | R | | L | | D | |
| | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ | k | $t_{1/2}$ |
| Jam 1 | 0.43 | 180 | 1.68 | 46.3 | 0.52 | 150 | 3.37 | 206 | 22.1 | 31.4 | 10.7 | 64.8 | 0.87 | 256 | 1.42 | 157 | 0.97 | 229 | 0.17 | 105 | 0.67 | 26.6 | 0.22 | 80.9 | 2217 | 35.1 | 3282 | 23.7 | 2766 | 28.1 |
| Jam 2 | 0.16 | 467 | 0.64 | 117 | 0.23 | 325 | 2.54 | 273 | 24.7 | 28.0 | 12.7 | 54.5 | 0.31 | 593 | 1.00 | 184 | 0.42 | 438 | 0.05 | 388 | 0.27 | 71.9 | 0.14 | 138 | 1755 | 118 | 2642 | 78.7 | 1969 | 106 |
| Jam 3 | 1.08 | 93.4 | 3.93 | 25.7 | 1.49 | 67.7 | 8.55 | 81.1 | 30.0 | 23.1 | 9.55 | 72.6 | 0.45 | 606 | 3.41 | 79.9 | 1.48 | 184 | 0.28 | 78.8 | 1.13 | 19.5 | 0.41 | 53.8 | 100 | 478 | 252 | 189 | 186 | 255 |
| Jam 4 | 0.82 | 97.5 | 1.72 | 46.5 | 0.98 | 81.6 | 2.35 | 295 | 24.1 | 28.8 | 9.67 | 71.7 | 0.40 | 441 | 1.60 | 110 | 0.70 | 251 | 0.17 | 103 | 0.65 | 26.8 | 0.25 | 69.7 | 1740 | 38.2 | 2542 | 26.2 | 2382 | 27.9 |
| Jam 5 | 0.20 | 485 | 0.74 | 131 | 0.39 | 249 | 6.89 | 101 | 46.7 | 14.8 | 35.8 | 19.4 | 0.21 | 479 | 0.75 | 134 | 0.26 | 387 | 0.07 | 197 | 0.23 | 60.0 | 0.10 | 138 | 1062 | 92.6 | 2835 | 34.7 | 1784 | 55.1 |
| Jam 6 | 0.11 | 586 | 0.37 | 174 | 0.34 | 190 | 4.75 | 146 | 29.9 | 23.2 | 7.83 | 88.5 | 0.38 | 255 | 1.73 | 55.9 | 0.96 | 101 | 0.10 | 106 | 0.45 | 23.7 | 0.12 | 88.8 | 349 | 229 | 1116 | 71.6 | 979 | 81.6 |
| Jam 7 | 0.59 | 124 | 2.81 | 26.2 | 2.21 | 33.3 | 4.43 | 156 | 34.5 | 20.1 | 11.8 | 58.6 | 0.46 | 353 | 2.04 | 79.6 | 1.41 | 115 | 0.10 | 139 | 0.51 | 27.2 | 0.13 | 107 | 348 | 233 | 1114 | 72.8 | 985 | 82.3 |

reported in a previous study (Pérez-Ramírez et al., 2015) indicating that incorporation of stevia could decrease the degradation rate of TPC during accelerated storage conditions. The effect of coconut sugar on phenolics degradation has not been previously reported; therefore, further research must be undertaken in order to elucidate the stabilizing effect of coconut sugar upon TPC during storage at 4 °C.

Interestingly, the half-life of TPC increases by almost 2.5 times in the case of lingonberry jam with erythritol stored under refrigeration compared to the control jam prepared with sucrose (Table 2). In another study (Nowicka & Wojdyło, 2016), in the presence of erythritol only 7 % of phenolics were degraded at 4 °C, suggesting a stabilizing effect of this sweetener on TPC. However, increasing the temperature and exposure to light resulted in an obvious destabilizing effect of erythritol, leading to a decrease of $t_{1/2}$ for phenolics from 605 days to 79.9 days. The only sweetener that maintains its effect on the phenolics stability both under light and higher temperature is coconut sugar (Table 2). The degradation in the presence of coconut sugar at 25 °C under dark occurred only 1.23-fold faster compared to the refrigerated jam, indicating a protective effect of this sweetener regardless temperature storage. In the case of all studied lingonberry jams, TPC degrades much slower than TAC, regardless of temperature and exposure to light, indicating a higher stability of these bioactive compounds during storage. Incorporation of other sweeteners than sucrose and lower storage temperature may decrease the degradation rate of TPC.

*Antioxidant capacity (AC)*

The results from this study illustrate that changes in AC are best fitted to a second-order kinetic model (Fig. 2), with $R^2$ values ranging from 0.926 to 0.995, this model being confirmed by other research (Molaveisi et al., 2019).As reported for previously bioactive compounds, temperature and exposure to light are the main factors that influence the degradation rate of antioxidants (Table 2). The storage at 25 °C results in a higher degradation rate of antioxidant compounds for all jam samples, especially in the case of jams formulated with erythritol, sucrose and brown sugar, respectively. The k values for degradation of antioxidants in sucrose jam increases with increasing temperature almost 4 times, from 0.280 $\cdot 10^{-3}$ $day^{-1}$ at 4 °C to 1.13 $\cdot 10^{-3}$ $day^{-1}$ at 25 °C exposed to light. Therefore, it can be concluded that antioxidants degrade slower under refrigeration and faster at higher temperatures (25 °C).

Following 180 days of storage at 4 °C, the changes in the AC of jams formulated with fructose, coconut sugar and stevia results in the slowest degradation, indicating the protective effect of these sweeteners. Contrariwise, the use of erythritol, sucrose and brown sugar accelerate the degradation process. The stability of antioxidants in the presence of fructose and coconut sugar is 5.60-fold and 4.00-fold, respectively, higher than that observed by storage in the presence of erythritol. When stevia is used as a sweetener, the degradation under refrigeration occurred almost 3-fold slower than in the case of erytrithol jam.

Antioxidants degradation during storage reflects the main trends observed in the changes of TPC ($R = 0.8974$). Coconut sugar, stevia and fructose are the sweeteners that resulted in the slowest degradation during the whole period. In both cases, the loss of AC follows second-order kinetics such as degradation of phenolic compounds. Moreover, half time values ($t_{1/2}$) decrease as the storage temperature increased in the same way with the degradation of TPC. Thus, the AC of the lingonberry jams may be due to the phenolic compounds.

*Reducing sugars*

Increasing the content of reducing sugars in jams during storage is best described by a zero-order kinetic model, with high $R^2$ value ranging from 0.793 to 0.992, which is in agreement with the results reported by others (Wibowo et al., 2015; Miguel et al. 2018). The estimated kinetic parameters are listed in Table 2. The use of different sweeteners in jam formulation may influence the accumulation rate of reducing sugars. The sucrose and brown sugar based jams present the highest accumulation rate regardless of storage conditions. A similar behavior was

previously reported for orange juice, which could be attributed to sucrose hydrolysis (Kennedy et al., 1990). This significant increase could be also linked to the increase in °Brix values during storage (Scrob et al., 2021). Light exposure resulted in increasing of the k value in the case of all jams, indicating a rapidly accumulation of reducing sugars in the presence of light. Increasing of reducing sugars under light could be also related to increasing of TSS content that was more visible in the case of samples stored at 25 °C, exposed to light conditions than those stored under refrigeration (Scrob et al., 2021). Temperature also plays an important role in reducing sugars changes. As expected, the accumulation rate increases by temperature. By comparing the half-life values (Table 2), it can be observed that at 25 °C (both under light and dark conditions), reducing sugars accumulate faster than under refrigeration, regardless of sweetener agent. This is in accordance with a study reported by Wibowo et al. (2015), which found that sugars accumulation was enhanced by an increase in storage temperature.

It is interesting to note that, regardless of storage conditions, the accumulation of reducing sugars is the lowest in erythritol jam (at least 10 times lower) followed by stevia and saccharin jam (which have almost identical accumulation rate), which suggests that these jams would be indicated in patients with diabetes. If in the case of erythritol jam it was found to have lowest values for sensory properties (Benedek et al., 2020; Scrob et al., 2021), in the case of the other two jams it was found to be equally preferred, stevia jam being favored due to its natural sweetener, with fewer side effects than artificial sweetener saccharin (Sclafani et al., 2010).

### *Statistical evaluation of nutritional and functional properties of lingonberry jams*

To identify specific patterns in stability process during storage, determined parameters (Vitamin C, TAC, TPC, AC and reducing sugars) were analyzed by applying the FA method and factorial ANOVA. The FA results show that the first three factors describe about 92.15 % – 93.19 % from the variability of determined parameters. Regardless of the storage conditions, the scores plots graph of the first 3 factors show the same classification of the samples (Fig. 3). Examine the rotated factor loadings (after a varimax rotation) (Table S1) the variables that has the most influence on each factor can be identified. Loadings close to −1 or 1 indicate that the variable strongly influences the factor. Loadings close

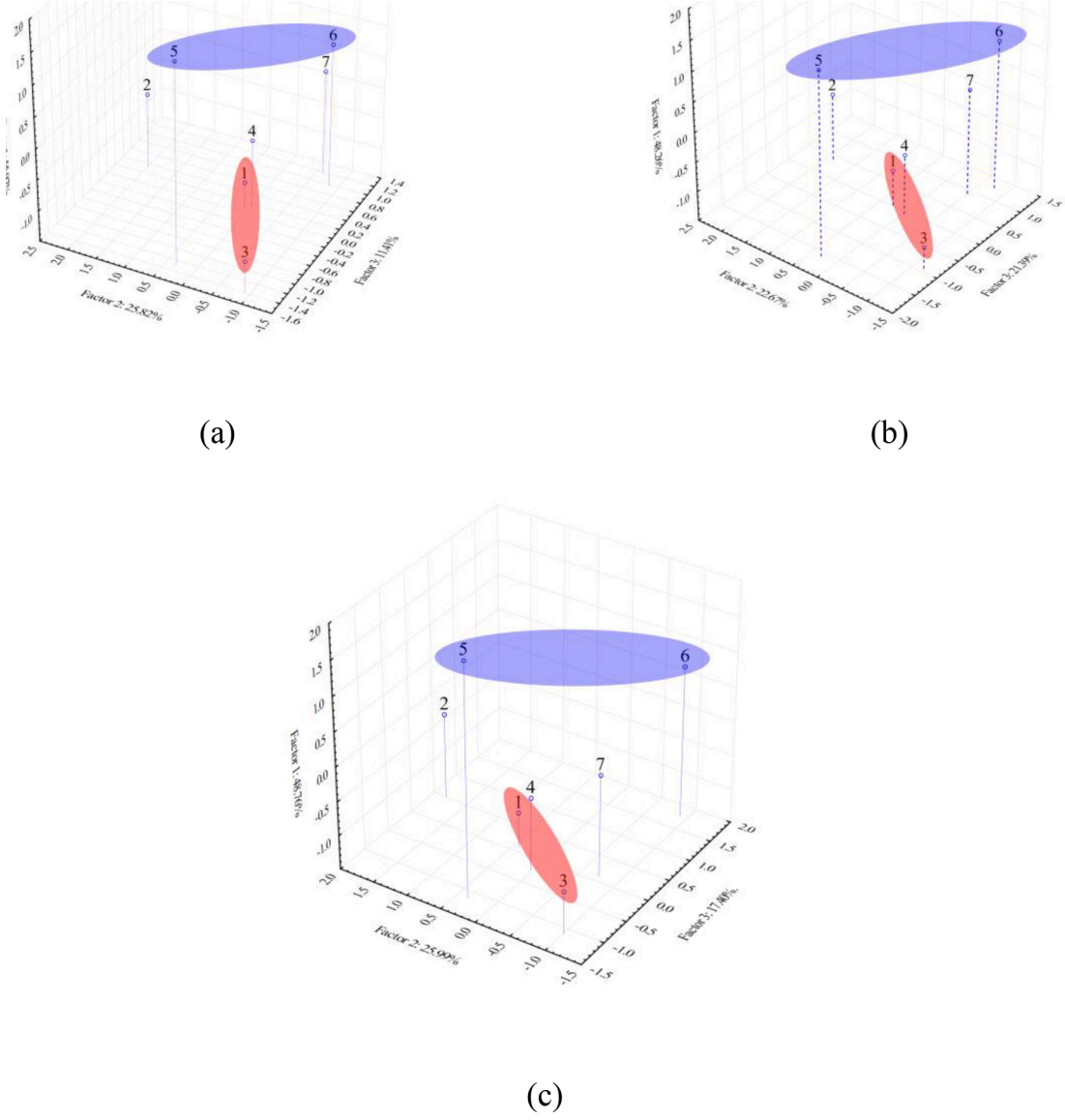


**Fig. 3.** Classification of the jam samples based on the variation of the analyzed parameters during 180 days under different storage conditions (refrigerator (a), dark (b) and light (c)): 1-sucrose; 2-fructose, 3-erythritol; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharin.

to 0 indicate that the variable has a weak influence on the factor. TPC (0.93 – 0.98, refrigerator conditions; 0.72–0.98, dark conditions; 0.85–0.95, light conditions) and AC (0.74–0.84, refrigerator conditions; 0.82–0.95, dark conditions; 0.83–0.91, light conditions) have large positive loadings on Factor 1 (55.97 %; 48.26 %; and 48.76 % from data variability), so this factor clearly describes the antioxidant potential of samples and stability of these components during storage. Sugars (0.92 – 0.98, refrigerator conditions; 0.91–0.99, dark conditions; 0.86–0.98, light conditions) have large positive loadings on Factor 2 (25.82 %; 22.67 %; and 25.99 % from data variability) and so this factor describes the sugars content during storage. Based on the similar considerations, Factor 3 (11.41 %; 21.39 %; and 17.40 %from data variability) describes the variation of the TAC with large positive loadings for refrigerator and dark condition respectively (0.74–0.93 and 0.78–0.98) and the vitamin C variation under light conditions (0.81–0.97).

In all of the storage conditions (Fig. 3) samples with coconut sugar (5) and with stevia (6) addition are characterized by higher scores of Factor 1 which associate these samples with a good stability for TPC and antioxidants during storage. On the other hand the samples with sucrose (1) and with erythritol (3) are associated with lower scores of Factor 1 indicating a lower stability of TPC and antioxidants during storage. So, a positive influence of coconut sugar and stevia addition can be considered regarding the TPC and other antioxidants stability. In addition, taking in consideration the above association of Factor 3 with stability of TAC and of Factor 2 with sugars, the stevia addition in the jam samples (6) reveals the higher stability of anthocyanin and vitamin C and lower increase of sugars.

Results are sustained also by degradation parameters obtained from experimental data (Table 2). The factor analysis performed on the kinetic data shows that the studied jam samples are described in proportion of 88.91 % by the first three factors (Table S2).

AC (0.91–0.95), vitamin C (0.82; 0.89) for refrigerator and light conditions respectively and TPC (0.72) for light conditions, have large positive loadings on Factor 1 (54.61 % from data variability) indicating this factor directly associated with rate of degradation of these compounds (high loading values – high degradation rate). TAC for dark and light conditions have large negative loadings (-0.89 and −0.91 respectively) on Factor 2 (24.32 % from data variability) so this factor is inversely associated with degradation rate of anthocyanins (high loadings values – low degradation rate). Sugars have large negative loadings (from −0.92 to −0.95) on Factor 3 (9.98 % from data variability) indicating the inversely association of this factor with degradation of sugars (high loading values – low degradation rate).

The scores plot of the factors describing the degradation rate parameters (Fig. 4) reveals that stevia (sample 6) and fructose (sample 2) addition show a lower decrease in AC and lower degradation of vitamin C and TAC. While fructose addition had a positive effect on the degradation processes of samples, it is important to note that its addition in jam samples increase considerable the reducing sugars content during the storage.

Moreover, applying factorial ANOVA it was found that no interaction between sweetener type and storage regardless of the tested parameter, which means that each factor acts individually as source of the detected variation.

## Conclusions

Preservation of bioactive compounds, such as anthocyanins, vitamin C, phenolics or antioxidant compounds in lingonberry jams during storage is of commercial importance. The stability of these compounds was higher at lower storage temperature (4 °C), while increasing the temperature at 25 °C and exposure to light determined higher rate of the degradation processes. Kinetic parameters (rate constants and half time values) showed that the stability of bioactive compounds was highly influenced by the type of sweetener used in jam formulation. Erythritol determined the most pronounced degradation of vitamin C and anthocyanins at both investigated temperatures, while phenolics were better preserved in the presence of this sweetener at 4 °C. Regarding AC, fructose proved to produce the slowest degradation rate of antioxidants, while the degradation was fastest in erythritol containing jams. In turn,

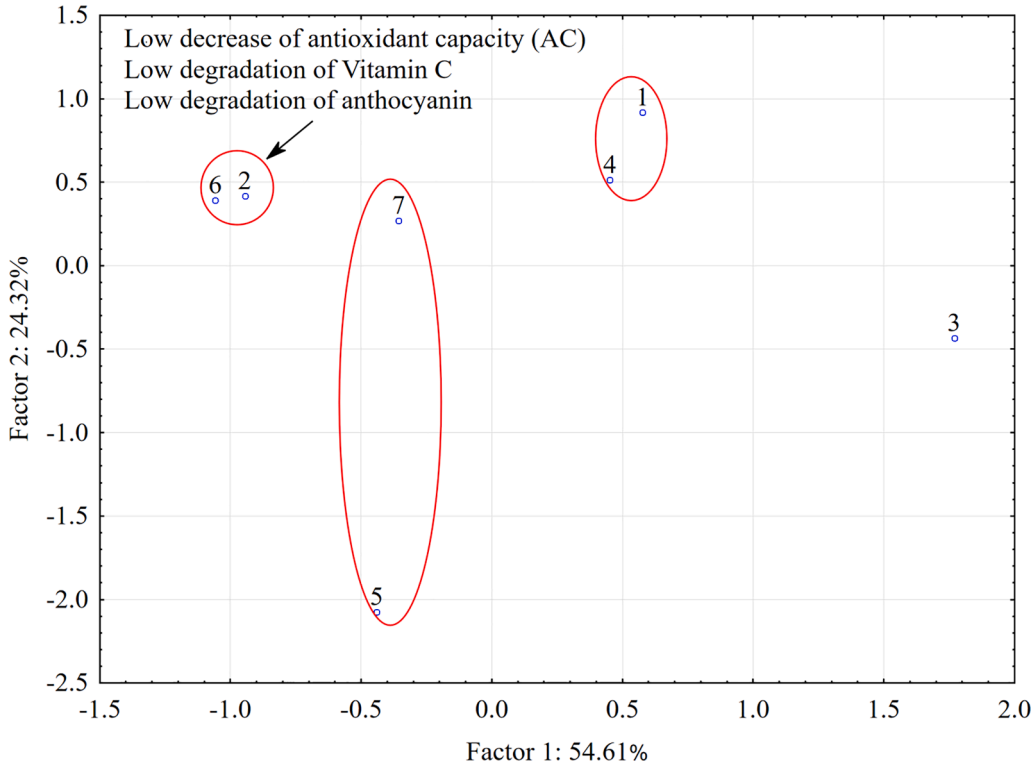


**Fig. 4.** Classification of the jam samples based on the degradation rate parameters during 180 days of storage: 1-sugar; 2-fructose, 3-erythritol; 4-brown sugar; 5-coconut sugar; 6-stevia; 7-saccharin.

the addition of coconut sugar resulted in lower degradation rate constants of phenolics, antioxidant compounds and vitamin C. The study also suggests that the addition of natural sweetener stevia enhanced the storage stability of most bioactive compounds, especially vitamin C.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors are unable or have chosen not to specify which data has been used.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100471.

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