

The change of phytochemical profile in beet juice and the influence of different storage conditions during one year

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Abstract

Many scientific researches proved the antioxidative impact of beet and its products. The use of vegetable juices is getting more popular in human diet. The way of storage plays an important role in preservation, long life and minimal variability of phytonutrients. The storage of products, usually in inappropriate conditions, leads to additional loss of phytonutrients, which have already been decreased by processing. In this research, we studied the impact of three ways of storage of pasteurised beet juice during one year, on content of some nutrients (total sugars, vitamin C, phenols and total antioxidative capacity). Pasteurised juice was stored in three ways: in light, at room temperature, in dark, at room temperature and in dark, at temperature of 4 °C. The change of content and differences have been followed during one month and confirmed with ANOVA and Tukey's test. The lowest changes of total sugars have been recorded in storage in dark at 4 °C, while in storage in light, the sugar content increased. The losses of vitamin C during one year of storage had linear trend of decrease. Antioxidative capacity of beet juice depends on concentration of phenol compounds and loss of these parameters was similar during period of one year. The best way of storage was dark place at low temperature.

Keywords: antioxidative complex; beet; juice; phenol; storage; sugar; vitamin C

Introduction

Beet belongs to a group of vegetables that has traditionally been used in human diet throughout the world and belongs to, so called functional food, since it contains bioactive compounds that have high benefit for human health (Paganga *et al.*, 1999; Clifford *et al.*, 2015). Some researches proved the positive impact of beet to certain diseases such as: hypertension, atherosclerosis, diabetes and dementia (Ninfali *et al.*, 2013). Among other strong antioxidants, beet root contains vitamin C, which neutralizes the free radicals in organism. However, vitamin C is thermally labile and is easily lost during thermal processing (Njoku *et al.*, 2011).

Received: 04 Jul 2022. Received in revised form: 05 Jul 2022. Accepted: 19 Jul 2022. Published online: 23 Sep 2022.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Significant part of total antioxidative activity of vegetables, besides vitamin C, depends on total phenol content (Obloh and Rocha, 2008). Also, studies proved that various beet processing impacts differently on phenol content in final product (Ravichandran *et al.*, 2012). According to Wruss *et al.* (2015) the antioxidative capacity of beet juice, stored for 7 months, differs among various types of beet and depends on concentration of phenol compounds. Total content of betalain was between 0.8 and 1.3 g/l in fresh juice (about 60% betacyanin and 40% betaxanthin) which was 70-100% part of total phenol content. Other phenols were hydroxymetal acids, which accounted up to 2.6% of total phenols.

Scientific studies proved many antioxidative, anti-inflammatory and chemo-preventing phytochemical activities of beet (*Beta vulgaris* ssp. *rubra*). Furthermore, it has beneficial impact on gastro-intestinal and cardiovascular system. Data shows that the beet extract can resist pro-inflammation cascades in mononuclear cells of periphery blood. Since the inflammation is strongly involved in development and progress of several clinical states, including the coronary disease and cancer, beneficial effect of beet juice extract can impact the anti-inflammatory capacity (Winkler *et al.*, 2005). Bioconversion of beet nitrates has an important role in blood pressure regulation, and red beet juice impacts iron metabolism. Fractioned beet juice, according to many researches, can be considered a good prevention from Alzheimer's disease (Babarykin *et al.*, 2019).

The use of vegetable juices and the mixture of vegetable and fruit juices are getting more popular in human diet. Thermal processing is the most common way of preservation (pasteurisation) that is used to inactivate the enzymes and microorganisms in order to increase the expiration date (Kathiravan *et al.*, 2015). Different recipes for food processing can have different effect to phyto chemical compound of product (Guldiken *et al.*, 2016). Generally, it is believed that the nutritional quality of food is lost after it has been thermally processed. However, sometimes food increases its quality after processing (Ravichandran *et al.*, 2012). The most important factors for this are time and temperature of processing (Guldiken *et al.*, 2016).

Given all the overall health benefits of consuming polyphenols, juices that contain high levels of this phytonutrient can be considered a positive dietary supplement. Beetroot juice is a significant source of dietary polyphenols (Wootton-Beard and Rya, 2011).

The goal for obtaining a healthy beet juice was to maintain the level of bioactive components at the initial level throughout the whole year. The maintenance of juice quality for the certain amount of time with the minimal loss of phytonutrients can be solved by the conservation method. In this way the level of microorganisms that cause the spoilage with light decomposition of pigments, is decreased, so one of the solutions might be the appliance of high pressure in preserving the beet juice (Sokołowska *et al.*, 2017).

The way of storage plays an important role in preservation, long life and minimal variability of phyto nutrients. Inadequate storage leads to additional loss of phyto-nutrients, which have already been decreased by processing. The preservation of phyto-nutrients in conditions imposed by market and the distribution to the consumer, challenges the researchers for inventing new ways of preservation. In this research, the impact of three ways of storage of pasteurised beet juice, during one year period and the level of some nutrients was investigated.

Materials and Methods

In order to research the stability of beet juice during one year, a beet (variety 'Palanačka crvena') from autumn production (August-October) was used. The beet was produced by using standard technology for post-harvest crop production in Serbia.

The juice was obtained by maceration of roots in centrifugal (rotational) juicer (Iskra, Slovenia), by separating liquid part from the pulp. The juice was pasteurised for 15 minutes at 90 °C temperature.

The comparison sample was the average value of pasteurized beet juice stored in 10, 250 mL, glass bottles per replication for each analysis – for every month. The trial was set in 3 replications.

Pasteurised juice was stored in three ways: in light – at room temperature, in dark – at room temperature and in dark – at temperature 4 °C. Sugar level, vitamin C, total phenols and total antioxidative activity in juice during year have been tested. The calendar dates of testing were: 10/03/2020; 10/05/2020; 10/07/2020; 07/09/2020; 10/11/2020; 10/01/2021; 10/03/2021.

Sugars

The level of sugars was determined by Bertran method. This method is used to determine all carbohydrates with free hemiacetal groups, which reduce metal ions (Cu^{2+} to Cu^+ from reagents Bertrand I ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)). The quantity of formed copper (I)-oxide was equivalent to sugar level. The solution of Bertrand III ($\text{Fe}_2(\text{SO}_4)_3$) dissolves the resulting precipitate of copper (I)-oxide whereby it again returns to copper (II), and iron (III) is reduced to iron (II). Iron (II) ions formed in an acidic environment oxidise with equivalent quantity of KMnO_4 to iron (III), and manganese (VII) turns into manganese (II). Based on the amount of KMnO_4 consumed, the corresponding amount of sugar can be read from the table (Cvijović and Aćamović, 2005).

Determination of Vitamin C

Pale beetroot juice was obtained by pressing 100 cm³ of beetroot juice and mixed with equal quantity of (100 cm³) solution of a mixture of HPO_3 and glacial acid CH_3COOH . Then, the mixture was filtrated through creased filter paper. The first 5-10 cm³ of filtrated mixture was thrown away and the aliquot part was taken from the rest of the mixture for the further investigation. If necessary, the investigated sample was diluted with cooled boiled distilled water, so the aliquot part contained about 2 mg of ascorbic acid. The process of determining ascorbic acid in the sample: 10 cm³ of filtrated sample (containing 5 cm³ of juice and 5 cm³ HPO_3 and glacial acid CH_3COOH) was applied to three Erlenmeyer dishes using pipette. Each sample was titrated with Tilman's reagent (TR) solution until pale pink, for about 5 seconds. At the same time, solution of TR was titrated and blind tested until pale pink (Cvijović and Aćamović, 2005).

The content of ascorbic acid (mg/cm^3) = $(V - V_1) \times T \times 100/g$

V – cm³ of TR solution used for titration in trial testing

V₁ – cm³ of TR solution used in blind testing

T – titer solution TR ($\text{mg C}_6\text{H}_8\text{O}_6 / 1 \text{ cm}^3 \text{ TR solution}$)

g – juice volume in cm³ in aliquot part of sample

Total phenols content

Total phenols in the beetroot ethanol extracts 20 g beetroot juice, 100 cm³ in ethanol were estimated according to the Folin–Ciocalteu method (Singleton *et al.*, 1999). The extract was diluted to the concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of NaHCO_3 (7.5%). Aliquots were left for 15 minutes at 45 °C, and then the absorbance was measured at 765 nm with a spectrophotometer against a blank sample. Gallic acid (GA) was used to calculate the standard curve. The assays were carried out in triplicate; the results were the mean values $\pm$ standard deviations and expressed as mg of Gallic acid equivalents per gram of dry extract (mg of GA/g).

Total antioxidant activity

Determination of total antioxidant activity by DPPH method has been done spectrophotometrically (Xu *et al.*, 2010). 8 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) were dissolved in methanol (100 mL) to give a concentration of 80 g/mL. Serial dilutions were made from the stock solution (1 mg/mL) of extract. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 minutes to any reaction occurred, and the absorption was measured at 517 nm. Ascorbic acid was used as the reference standard and dissolved in methanol to make a stock solution with the same concentration of 1 mg/mL. The control sample was prepared

to contain the same volume, but without the test compound or reference antioxidants. 95% percent methanol was used as a blank. Three measurements were made.

Analysis data

Differences of the means (among the level of phyto-chemistry components in fresh fruits and products, the ratio in light, at room temperature, in dark, at room temperature and in dark, at 4 °C temperature) have been determined according to the analysis of variance - ANOVA model, and the significant difference was expressed by LSD test. Storage in three ambient conditions was analysed by the trend line, representing the average state of the observed phenomena through time, as follows (Njegić *et al.*, 1991):

$$\hat{y} = a + bx$$

Where:

$$b = \frac{\sum x_t y_t - n \cdot \bar{x} \cdot \bar{y}}{\sum x_t^2 - n \cdot \bar{x}^2}$$

$$a = \bar{y} - \bar{x} \cdot b$$

Results and Discussion

Sugars

Different level of sugar during one year was found for different ways of storage. The lowest changes were recorded in dark storage at 4 °C, while in light the sugar level increased (Figure 1). The significant changes were recorded in juices stored in dark comparing to light and statistically important difference was found in dark storage at room temperature and at 4 °C (Table 1).

The changes were linear under all storage conditions, as confirmed by the significant values coefficient of determination ($R^2 = 0.9786$; $R^2 = 0.8012$). The lower coefficient of determination ($R^2 = 0.6972$) indicate a decrease in total sugars during one year of storage in light conditions (Figure 5-A).

The stability of sugar content in beet juices obtained from different beet varieties, after seven months of storage in dark place was researched by Wruss *et al.* (2015). The level of sugar in their research was 7.7%. Our results of average sugar level when stored in dark place were in accordance with results obtained by Wruss *et al.* (2015), where the storage at room temperature during one year remained unchanged.

Table 1. Tukey's Multiple Comparison Test, for the dynamics of sugar level change during one year of storage

Tukey's Multiple Comparison Test	Mean	q	Significant p < 0.05	95% CI of diff
light: dark	0.514	3.111	ns	-0.082 to 1.111
light: dark 4 °C	1.129	6.827	**	0.532 to 1.725
dark: dark 4 °C	0.614	3.716	*	0.018 to 1.211

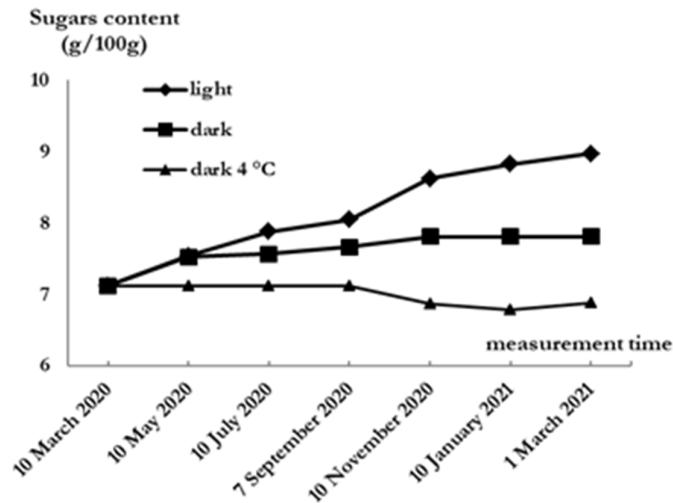


Figure 1. Average value of sugar content in beet juice during one year storage

Vitamin C

The greatest losses of vitamin C were found in juices stored at light. Storage in dark was significantly different comparing to storage in light for period of one year. No significant differences were found for storage at both temperatures (dark and dark 4 °C) (Figure 2, Table 2).

The average values of vitamin C decreased. The losses during one year of storage decrease linearly with high coefficient of determination, light, dark and dark 4 °C ($R^2=0.9972$, $R^2=0.9034$ and $R^2=0.9654$) Figure 5 (B).

Concentration of vitamin C in fresh beet root can vary, depending on cultivation, growing conditions etc (Leong and Oey, 2012; Szopinska and Gaweda, 2013). Our results were in accordance with Njoku *et al.* (2011), Adefegha and Oboh (2011), who proved that the processing temperature directly impacts the denaturation of vitamin C, as well as the decrease of average value in product during one year of storage (Pavlović *et al.*, 2019).

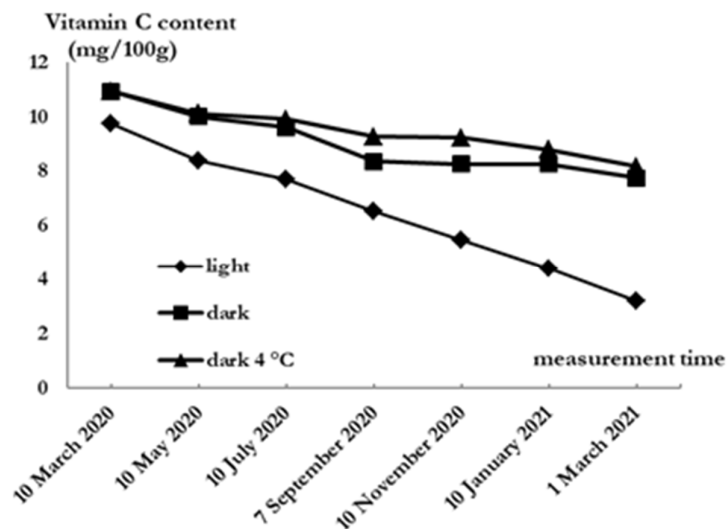


Figure 2. The average values of vitamin C content in beet juice during one year

Table 2. Tukey's Multiple Comparison Test, for the dynamics of the change of content of vitamin C during one year of storage

Tukey's Multiple Comparison Test	Mean	q	Significant p < 0,05	95% CI of diff
light: dark	-2.571	3.949	*	-4.922 to 0.221
light: dark 4 °C	-3.000	4.607	*	-5.350 to 6.499
dark: dark 4 °C	-0.428	0.658	ns	-2.779 to 1.922

Phenols

During one year of storage of beet juice a significant loss of total phenols was found. The loss was different in different conditions of storage. The highest losses were recorded for storage in light. The highest losses were found when preserved at light. The lower losses were found for storage in dark, especially when stored in refrigerators, while storage in dark in non-acclimatised conditions was good in the first three months and after that period the level of total phenols abruptly dropped. In the following period, the downward trend continued, but in a less dynamic decline (Figure 3).

Significant differences were recorded when stored at light and for both ways of storage in dark. Storage in dark in two conditions wasn't significantly different, according to Tukey's Multiple Comparison Test (Table 3).

The trend of decrease of average value of phenol content in beet juice was proved by decreasing line trend with high coefficient of determination (R^2) (Figure 5 (C)). These results were in accordance with results of Kujala *et al.* (2000), who found significant differences in total phenol content when stored at 5 °C during period 0 – 196 days. The level of phenols in bio-fermented juices also decreased when stored at low temperatures. Total level of phenols decreased during storage process and was 540 mg / L (Czyżowska *et al.*, 2020).

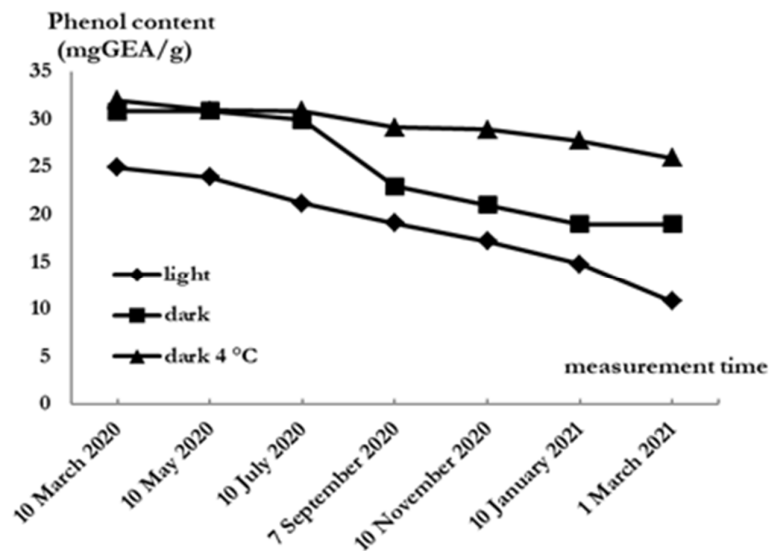
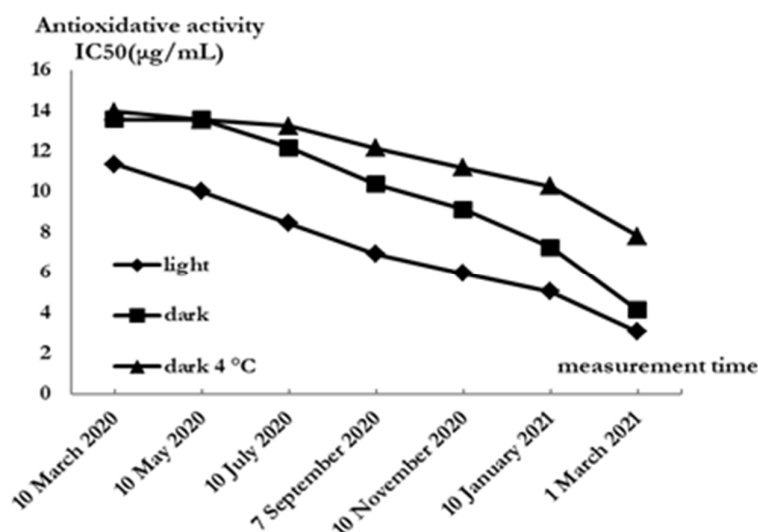
**Figure 3.** The average values of phenol level in beet juice during one year

Table 3. Tukey's Multiple Comparison Test, for the dynamics of change of phenol level during one year of storage

Tukey's Multiple Comparison Test	Mean	q	Significant p < 0,05	95% CI of diff
light: dark	-6.143	3.665	*	-12.19 to -0.094
light: dark 4 °C	-10.71	6.392	**	-16.76 to -4.665
dark: dark 4 °C	-4.571	2.727	ns	-10.62 to 1.478

Total antioxidant activity

Total antioxidative activity of beet juice during one year of storage dropped. The highest loss was found for storage at light, while the storage in dark at room temperatures only slowed down the loss, but after one year it had only slightly higher antioxidative activity comparing to storage in light. Slower decrease of average values of antioxidative activity was determined in juice stored in dark at 4 °C. There was a significant difference between storing in dark at 4 °C and storing at light place (Figure 4, Table 4).

**Figure 4.** The average values of total antioxidant activity in beet juice during one year of storage**Table 4.** Tukey's Multiple Comparison Test for the dynamics of change of total antioxidative activity during one year of storage

Tukey's Multiple Comparison Test	Mean	q	Significant p < 0,05	95% CI of diff
light: dark	-2.857	2.549	ns	-6.902 to 1.188
light: dark 4 °C	-4.571	4.079	*	-8.616 to -0.526
dark: dark 4 °C	-1.714	1.529	ns	-5.759 to 2.331

Tendency of loss was highly determined by coefficients of determination (R^2), light, dark and dark 4 °C; $R^2 = 0.9921$, $R^2 = 0.9454$ and $R^2 = 0.9155$ (respectively), Figure 5 (D).

High temperatures during pasteurisation, according to Boari *et al.* (2013) caused losses of antioxidative activity for 5.5% of dry matter. This loss happened during the processing. Similar point of research had Sawicki and Wiczowski (2018). They proved that after thermal treatment the profile, content and antioxidative

capacity of the beet juice modulated. Our results were in accordance with Guldiken *et al.* (2016), who proved that thermal processing of beet decreases the antioxidative activity. The same results were presented by Fang *et al.* (2008) when investigating the thermal processing of mustard. However, Adefegha *et al.* (2011) proved that cooking of plant material increases antioxidative activity. Our research proved additional decrease of average value of total antioxidative activity during storage in different ratio, depending on storage conditions.

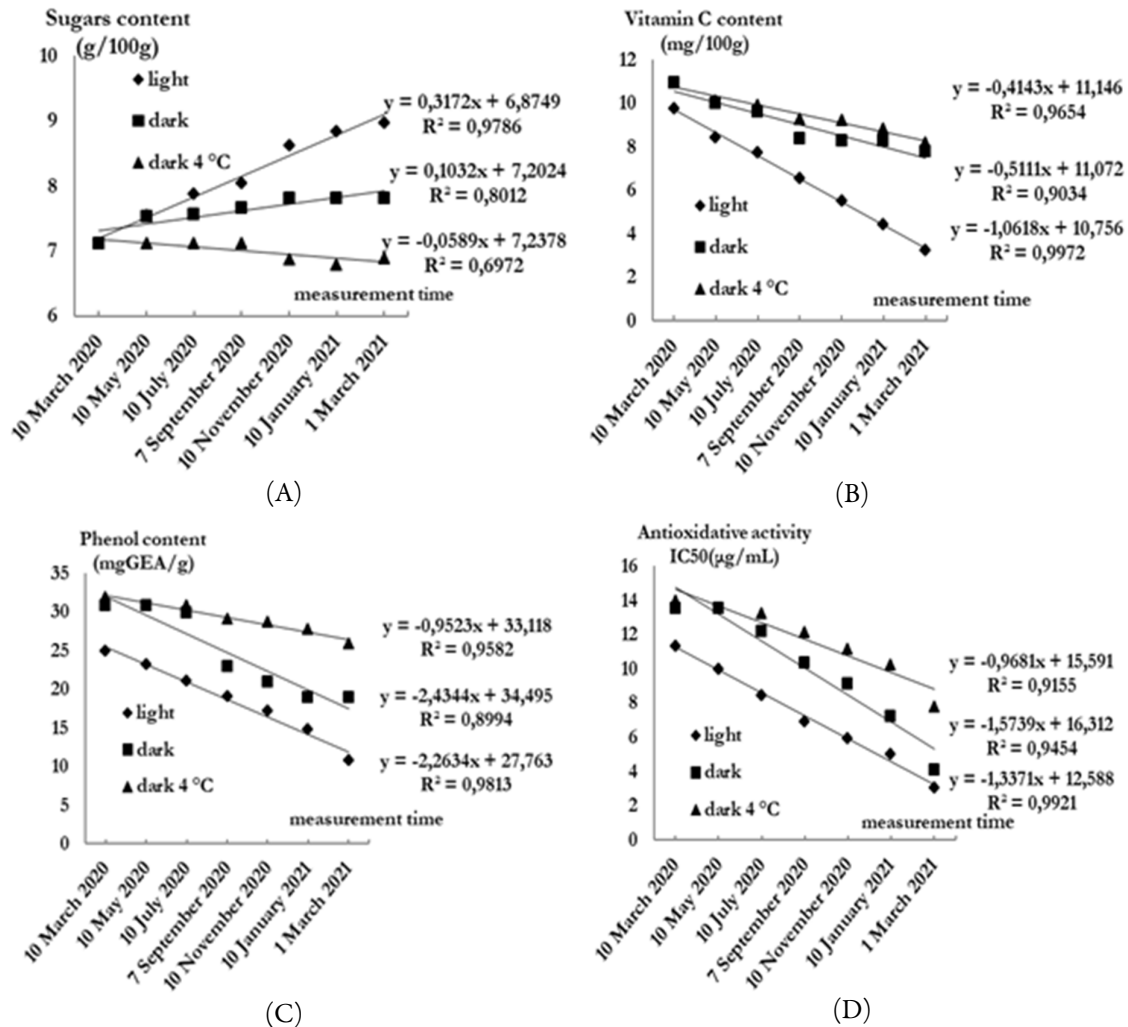


Figure 5. Tendency of change of bioactive components during one year of storage: sugar (A); vitamin C (B); phenols (C); total antioxidative activity (D)

According to Wruss *et al.* (2015) the antioxidative capacity of beet juice depends on concentration of phenol compounds. Our results proved the same, since the changes during storage for both researched factors (total level of phenols and total antioxidative activity) were the same during whole period of storage, regardless to conditions. The decrease for both researched factors tended for linear flow (Figure 5, C and D).

Conclusions

The lowest changes in total level of sugar in beet juice were found in storage in dark place at 4 °C. Vitamin C losses during a year of storage. Also, during the one year of storage in beet juice, loss of total phenols and antioxidative capacity.

Authors' Contributions

Conceptualization (MZ and ĐM); Investigation (MM); Methodology (JM); Software (DT); Writing - original draft (NP); Writing - review and editing (JZ). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The research was financed by the Ministry of Education, Science and Technological Development Republic of Serbia, project ref, number 451-03-68/2022-14 and 451-03-68/2022-14/200217.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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