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).
Significant part of total antioxidative activity of vegetables, besides vitamin C, depends on total phenol content (Oboh 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 hydroximetal 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 (Sokolowska 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; 10/06/01/03/2021.

**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 (CuSO$_4$.5H$_2$O)). The quantity of formed copper (I)-oxide was equivalent to sugar level. The solution of Bertrand III (Fe$_2$(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 cm$^3$ of beetroot juice and mixed with equal quantity of (100 cm$^3$) solution of a mixture of HPO$_3$2 and glacial acid CH$_3$COOH. Then, the mixture was filtrated through creased filter paper. The first 5-10 cm$^3$ 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 cm$^3$ of filtrated sample (containing 5 cm$^3$ of juice and 5 cm$^3$ HPO$_3$2 and glacial acid CH$_3$COOH) was applied to three Erlenmeyer dishes using pipette. Each sample was titrated with Tilmans 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).

\[
\text{The content of ascorbic acid (mg/ cm}^3\text{) = \left( V - V_1 \right) \times T \times 100/g}
\]

\[
V - \text{cm}^3\text{ of TR solution used for titration in trial testing}
\]

\[
V_1 - \text{cm}^3\text{ of TR solution used in blind testing}
\]

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

\[
g - \text{juice volume in cm}^3\text{ in aliquot part of sample}
\]

**Total phenols content**

Total phenols in the beetroot ethanol extracts 20 g beetroot juice, 100 cm$^3$ 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 ± 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):

\[
y = a + bx
\]

Where:

\[
b = \frac{\sum x_i y_i - n \cdot \bar{x} \cdot \bar{y}}{\sum x_i^2 - n \cdot \bar{x}^2}
\]

\[
a = \bar{y} - \bar{x} \cdot b
\]

**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

<table>
<thead>
<tr>
<th>Tukey’s Multiple Comparison Test</th>
<th>Mean</th>
<th>q</th>
<th>Significant p &lt; 0.05</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>light: dark</td>
<td>0.514</td>
<td>3.111</td>
<td>ns</td>
<td>-0.082 to 1.111</td>
</tr>
<tr>
<td>light: dark 4 °C</td>
<td>1.129</td>
<td>6.827</td>
<td>**</td>
<td>0.532 to 1.725</td>
</tr>
<tr>
<td>dark: dark 4 °C</td>
<td>0.614</td>
<td>3.716</td>
<td>*</td>
<td>0.018 to 1.211</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tukey’s Multiple Comparison Test</th>
<th>Mean</th>
<th>q</th>
<th>Significant p &lt; 0.05</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>light: dark</td>
<td>-2.571</td>
<td>3.949</td>
<td>*</td>
<td>-4.922 to 0.221</td>
</tr>
<tr>
<td>light: dark 4 °C</td>
<td>-3.000</td>
<td>4.607</td>
<td>*</td>
<td>-5.350 to 6.499</td>
</tr>
<tr>
<td>dark: dark 4 °C</td>
<td>-0.428</td>
<td>0.658</td>
<td>ns</td>
<td>-2.779 to 1.922</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Tukey’s Multiple Comparison Test</th>
<th>Mean</th>
<th>q</th>
<th>Significant p &lt; 0,05</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>light: dark</td>
<td>-6.143</td>
<td>3.665</td>
<td>*</td>
<td>-12.19 to -0.094</td>
</tr>
<tr>
<td>light: dark 4 °C</td>
<td>-10.71</td>
<td>6.392</td>
<td>**</td>
<td>-16.76 to -4.665</td>
</tr>
<tr>
<td>dark: dark 4 °C</td>
<td>-4.571</td>
<td>2.727</td>
<td>ns</td>
<td>-10.62 to 1.478</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tukey’s Multiple Comparison Test</th>
<th>Mean</th>
<th>q</th>
<th>Significant p &lt; 0,05</th>
<th>95% CI of diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>light: dark</td>
<td>-2.857</td>
<td>2.549</td>
<td>ns</td>
<td>-6.902 to 1.188</td>
</tr>
<tr>
<td>light: dark 4 °C</td>
<td>-4.571</td>
<td>4.079</td>
<td>*</td>
<td>-8.616 to -0.526</td>
</tr>
<tr>
<td>dark: dark 4 °C</td>
<td>-1.714</td>
<td>1.529</td>
<td>ns</td>
<td>-5.759 to 2.331</td>
</tr>
</tbody>
</table>

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 Wiczkowski (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.

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).

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

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Conflict of Interests

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

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