SUGAR AND ACID CONTENT OF ORGANICALLY AND CONVENTIONALLY GROWN STRAWBERRY (FRAGARIA × ANANASSA)

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Abstract
In a series of experiments we investigate the effect of growing conditions on the quality of strawberry fruits. In the present article, carbohydrate and organic acid components of fruits grown under organic conditions versus conventional farming conditions were compared. Effects of two biostimulants licensed for organic farming were also tested. We found that sucrose:fructose ratio was lower in organic fruits. Weather conditions were unfavorable in 2019 for strawberry production therefore sugar concentrations were much lower than expected.

1 Introduction
Based on WHO data, 60 million people suffers diabetes in Europe [14]. In Hungary 13 % of adults and 0.27 % of children (0-18 years old) are diabetic, based on 2017 data [10]. Sugar content and composition is especially important for diabetic patients, and people with insulin resistance. Glycemic index of strawberry is relatively low. The favorable distribution of carbohydrates (content of sucrose is low while the content of fructose is higher) makes it edible for diabetics as well. Total sugar content of strawberry fruits is about 8.6 g / 100 g [3].

Organic acids are important health-protecting components. Consumption of organic acids has a metabolic stimulating effect and it can reduce the complaints of people suffering from gout or rheumatism [4]. On the other side, people who react sensitively to fruit acids – and also breastfeeding mothers – might prefer fruits with low acid concentrations [4].

Results on the nutrient value of fruits grown organically versus conventionally are contradictory. In the publications comparing organic versus conventional conditions, farms are on different locations, and therefore weather conditions, soil type etc. are different, so it is difficult to separate the effects of the many complex factors [7] [13]. To get reliable picture on the differences, as a first step we applied an experimental design, where on the same location different nutrient supply methods (inorganic versus organic fertilizers) were applied. In a series of experiments we compare the yield and quality (dry matter content, macro- and microelements, sugar, acid, antioxidant, etc.) of strawberry fruits applying organic versus inorganic nutrient supply.

Later on further factors will be tested. If we can approach the yields of conventional farming and prove that nutrient values and health-promoting compounds exceed those of conventional fruits, it may be worth considering organic strawberry production, as it can be consumed as „functional food“.

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In the present work, we compare the most important sugar and organic acid compounds of fruits, harvested in the year of planting.

Algae biostimulants improve photosynthetic activity, therefore they might increase carbohydrate content of strawberry. PGPR bacteria help nutrient intake of plants on a number of way, which can stimulate their biochemical pathways, their metabolism [11], [12]. Because of the above reasons and because microbial products are more and more used in organic crop production [5], two microbial products were involved in the experiment.

2 Material and Methods

2.1 Experimental set up, cultivar, planting, culture conditions

Our experiment was set up at the Experimental Garden of the Faculty of Horticulture and Rural Development of John von Neumann University (Kecskemét, Hungary). *Fragaria × ananassa* ‘Asia’ frigo seedlings were planted on 26th of March 2019 in a twin-row arrangement (30 * 25 cm + 60 cm cultivation path). 4 twin rows plus one- one single row on both sides were planted. 8m * 4 m area, treatments were separated by 0.5 m fallow strips.

4 different culture conditions (nutrient supply were tested):
- ORG: organic fertilizers were applied
- ORG+B: organic fertilizers + Bacterial inoculation
- ORG+B+A: organic fertilizers + Bacterial inoculation + Alga suspension
- CONV: inorganic fertilizers

Basic fertilization and nutrient replenishment were planned based on soil analysis. As this is a comparative experiment, the nutrient replenishment was designed so, that approximately the same amount of nitrogen and potassium would be applied in the organic and conventional fields. Accordingly, a total of 233 kg / ha of Haifa Turbo and 125 kg / ha of granular potassium sulphate was applied to the conventional plot, while 700 kg / ha of Phoenix pelleted poultry manure was applied to the organical area, so that the same amounts of nitrogen and potassium were applied. Irrigation was performed with a microspray irrigation system.

Due to several weeks of rain in early May, fungal control became necessary. On 31 May, copper-containing foliar fertilizer (Scudo) preparation was applied at a concentration of 2 ml / l.

2.2 Bacterial inoculations

On one plot a bacterial soil inoculant (BactoFil B-10) was applied (ORG+B). The product was diluted with tap water, sprayed on the soil and worked in immediately. Inoculation was executed directly before planting. Besides, 100 ml of 5000 x diluted BactoFil B-10 was poured in every planting hole, right before planting of frigo transplants.

2.3 Treatment with alga suspension

A microbial product (Algafix), containing *Scenedesmus obtusiusculus* cells was diluted with tap water and sprayed on the plants in 2 l / ha quantity at the beginning of flowering.

2.4 Chemicals applied for analysis

Citric acid (98% purity), malic acid (95% purity), tartaric acid (96% purity), succinic acid (99% purity), glucose (99,5% purity), fructose (99% purity), sucrose (99,5% purity) were purchased from Sigma-Aldrich. Sulfuric acid (98%) was purchased from Merck. The high purity water was produced in the analytical laboratory of Széchenyi István University.

2.5 Sample preparation

Fruits were picked randomly from 20-20 plants / treatment. In order to avoid border effect, no samples were taken from the single rows. For the extraction, raw samples were used. As a first step, the fruits were washed and the parts suitable for human consumption chopped with a kitchen chopper (Old Smith) (parts size less than 1mm) and then standardized.
Samples were measured using precision tare balance (Denver Instrument, type APX-3202). 1-1 grams of samples (3-3 parallel measurements per sample) were weighed into 250 ml Erlenmeyer flasks and 50 ml of high purity water was added. Extraction was performed using 358 S-type (Elpan) laboratory shaker. Samples were shaken for one hour, and 10ml of each sample were put into a 15ml centrifuge tube. The centrifugation was performed with a Z 206 A type centrifuge (Hermle) at 6000 rpm for 30 min. After centrifugation, 1-1 ml of supernatants were filtered into 1.8 ml screw capped vials trough 0.22 µm hydrophilic syringe filter.

2.6 HPLC analysis

2.6.1 Analysis of organic acids

The organic acids were analyzed by ion exclusion chromatography, with a Jasco HPLC system (Jasco LC-900 series). A Bio-Rad Aminex HPX-87H column was used to separate the constituents. The column temperature was 35 °C. 0.1% sulfuric acid was used as eluent and the flow rate was 0.5 ml / min. The detection wave-length was 210 nm.

2.6.2 Analysis of sugars

Sugar contents were analyzed by ion exclusion chromatography, using the Jasco HPLC system (Jasco LC-900 series). Supercogel H type (Sigma-Aldrich) column used for the separation at room temperature. High purity water was used as eluent with a flow rate of 0.5 ml / minute. Refractive index detector was used to determine the quality and quantity of the components.

3 Results and Discussion

3.1 Organic acid content of fruits

Concentrations of 4 organic acids (malic acid, citric acid, tartaric acid and succinic acid) were measured. In our samples no tartaric and succinic acid were found. Concentrations of malic and citric acids are summarized in Table 1.

Table 1. Organic acid concentrations of strawberry fruits grown organically versus conventionally in mg / g fresh weight. Means ± Standard deviations, n=3. The different treatments marked as: ORG: nutrient supply according to organic farming B: organic farming+ BactoFil B-10 treatment B+A: organic farming+ BactoFil B-10 and Algafix treatments, CONV: conventional.

<table>
<thead>
<tr>
<th></th>
<th>mg/g FW</th>
<th>Malic acid</th>
<th>Citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORG</td>
<td>1.89 ± 0.05</td>
<td>6.64 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>ORG+B</td>
<td>1.35 ± 0.14</td>
<td>5.59 ± 0.29</td>
<td></td>
</tr>
<tr>
<td>ORG+B+A</td>
<td>2.15 ± 0.13</td>
<td>5.29 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>CONV</td>
<td>0.75 ± 0.1</td>
<td>6.67 ± 0.19</td>
<td></td>
</tr>
</tbody>
</table>

Our results are similar to the values published in the nutrition and nutrients composition book of [3]. We found that malic acid concentrations varied between 1.18 and 2.29 mg /g, while they published that general malic acid content of strawberry is about 1.5 mg /g. Concentrations for citric acid varied between 5.2 and 6.92 mg /g, while Bíró-Lindner [3] published the general value of 5 mg/g for strawberry. Neither tartaric nor succinic acids were detected in the samples, though based on the Bíró-Lindner book [3], general tartaric acid content of strawberry is about 0.6 mg /g. Organic acid concentrations showed no clear correlations with the applied treatments.
3.2 Carbohydrates of fruits

As the most abundant sugars in strawberry are glucose, fructose and sucrose [1] [3], our study focused on these carbohydrates.

Our results showed higher glucose, fructose and total sugar while lower sucrose level in fruits of organically-grown strawberry plants. We found no clear effect of the applied microbial products on carbohydrate levels.

In the literature, we find contradictory data on whether there is a significant difference in the carbohydrate composition and concentrations of organic versus conventional fruits. Cayuela et al., [6] and Conti et al., [7] found higher sugar content in organic fruits of strawberry, while others found no significant differences [8], [9] [13].

Table. 2. Average sugar concentrations of organically versus conventionally grown strawberry fruits. Means ± Standard deviations, n=3. The different treatments marked as: ORG: nutrient supply according to organic farming B: organic farming+ BactoFil B-10 treatment B+A: organic farming+ BactoFil B-10 and Algafix

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORG</td>
<td>13.04 ± 0.384</td>
<td>17.53 ± 0.497</td>
<td>0.127 ± 0.114</td>
<td>30.697</td>
</tr>
<tr>
<td>ORG+B</td>
<td>7.791 ± 0.757</td>
<td>11.35 ± 0.973</td>
<td>0.076 ± 0.065</td>
<td>19.217</td>
</tr>
<tr>
<td>ORG+B+A</td>
<td>8.278 ± 1.157</td>
<td>10.87 ± 1.479</td>
<td>0.063 ± 0.339</td>
<td>19.211</td>
</tr>
<tr>
<td>CONV</td>
<td>5.737 ± 1.033</td>
<td>9.252 ± 1.655</td>
<td>0.272 ± 0.188</td>
<td>15.261</td>
</tr>
</tbody>
</table>

As regards sucrose, our results are consistent with the findings of Akšić and co-workers. They found that organically-grown fruits contain less sucrose [1]. In our experiments sucrose: fructose ratio was about 0.01 in organic while 0.03 in conventional fruits. These findings are interesting, but carbohydrate metabolism and accumulation is very complicated and multi-factorial process, therefore we have to be careful when drawing conclusions.

One of the main factors influencing the nutritional composition of fruits is the maturation stage of the fruit. The carbohydrate concentrations measured in our study (Table 1.) are far below the concentrations known from literature. In the nutrition and nutrients composition book from Bíró–Lindner reference values for the different carbohydrates are 2-3 fold higher [3] compared to our results.

For correct interpretation of these data we have to take into account that 2019 was an extremely problematic year for strawberry cultivation. Large amounts of precipitation and the lack of sunshine in the ripening period caused problems. Constant humidity favored the occurrence of fungal diseases. Our plantation showed symptoms of Phytophthora and Colletotrichum fragariae, which resulted in significant stem destruction and an average yield loss of 16–32% (data not shown).

Due to the rainy weather and the poor sunshine period, we could observe that the nutritional parameters of the fruits did not develop favorably. This can be one of the reasons why our results on the different carbohydrate concentrations remain below the expected values for strawberries [1], [2] [9].

On other reason for the low carbohydrate concentrations could be, that as we did not want to lose more fruits due to fungal disease we probably picked fruits too early. As strawberry is a non-climacteric fruit has to be picked at full maturity. So in this case they could not reach the full maturity and therefore the nutritional and sensory qualities were not able to develop. Hopefully the results of the next growing season will be more reliable.

4 Conclusion

When interpreting the results, we have to take into account, that differences in the content of fruit produced under organic and conventional farming conditions are a consequence of several years of organic farming, thanks to healthy ecosystems in the area and soil microbiomes. Nutrient
supply is only one component of the different, complex factors effecting quality of fruits. 2019 growing season was extremely problematic for strawberry production, weather conditions, and fungal diseases influenced our results. It is expected that we will get more reliable results in the coming years.

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