MEASURING THE QUANTITY OF ANTHOCYANIDINS IN FROZEN FRUITS

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Abstract

Fruits are recommended in the everyday diet, the recommendation coming from the content of the fruits: antioxidants, minerals, water, dietary fibers. The first line behind the antioxidant status are anthocyanidins and vitamins with proven positive effects for the human well-being. Each fruit has its own fresh-available period, so it needs to be additionally treated in order to stay fresh during the whole year. Freezing is one of the options to be used for a longer period and to keep preserve all its healthy nutrients, which is not the case when it is thermally treated. Quick frozen fruits are identical for their nutritional content as the fresh ones.

We analyzed the following fruits during our research: raspberries, strawberries and sour cherries which are naturally high in flavonoids and anthocyanidins with very effective antioxidant and anticancer properties and protect the human body from cardiovascular, degenerative illnesses and cancers. In our research, the measuring in the frozen fruits is made with a spectrophotometric method - pH differential method.

It was established that the highest amounts of anthocyanidins are registered in the raspberries 94.45 mg/100 g, in the sour cherries 65.89/100 g and in the strawberries 35.67 mg/100g. The total acidity and pH level of the fruits are determined with this research. The biggest total acidity have the raspberries 16.73 g/100g with the determined the lowest pH level (3.90).

The freezing on low temperatures not just allows to prolong the period for consuming of the fruits, it also allows to preserve its nutritional quality, which can be seen from the results we got in our research.

Key words: Antioxidants, Healthy nutrients, Spectrophotometer, Total anthocyanidins, Frozen fruits, Raspberries, Strawberries, Sour cherries.

1. Introduction

Anthocyanins are water-soluble pigments produced via the flavonoid pathway in the cytoplasm of the colored plant cell. The attachment of the sugar molecule makes them particularly soluble in the sap of the vacuole, where these molecules are stored. These give them the pink-red colors of most flower petals, of most red fruits (like apples) and almost all red leaves during the autumn. They absorb light in the blue-green wavelengths, allowing the red wavelengths to be scattered by the plant tissues to make these organs visible to us as red [2].

Chemically anthocyanins are subdivided into the sugar-free anthocyanidine aglycons, the anthocyanin glycosides.

Figure 1. Spectral characteristics of purified radish anthocyanins (acylated pelargonidin-3-sophoroside-5-glucoside derivatives) in pH 1.0 and pH 4.5 buffers.
Anthocyanins may be distributed throughout the fruit, as is the case of raspberries, strawberries, and blackberries, or limited to the skins, as is the case of apples and most blueberry cultivars. Anthocyanins in most cultivars of cherries are present in skins and flesh, whereas in some cultivars of cherries they are limited to the skins. Structure, pH, temperature, light, oxygen, metal ions, intramolecular association, and intermolecular association with other compounds (copigments, sugars, proteins, degradation products, etc.), are generally known to affect the color and stability of anthocyanins. Frozen fruits constitute a large and important food group in modern diet. Fruit may be more extensively used if it is available during the off-season. Freezing also makes year-round further processing of fruit products, such as: jams, juice, and syrups, from frozen whole fruits, slices, or pulps possible [1].

The red and blue color of the fruit is related to its anthocyanin composition. Loss of fruits color during freezing, processing and storage has been attributed to many factors such as: enzyme reactions, ascorbic and other organic acids, sugar products, oxygen, fruit maturity, thawing time, metal ions, light, and temperature and also may be affected by the actual anthocyanin concentration [1].

Anthocyanins are relatively unstable during frozen storage and are greatly affected by: pH, organic acid content, sugar concentration, enzyme reactions, and anthocyanin content.

Changes in antioxidant content and color take place in frozen berries as a result of oxidation reduction reactions occurring in fruits [3].

These changes are influenced by:
- Initial quality of berries.
- Raw material processing prior to freezing.
- Freezing methods.
- Storage conditions (temperature and relative humidity).
- Storage time of frozen berries and quality of packaging.

The chemical basis for these desirable properties of flavonoids is believed to be related to their antioxidant capacity - their ability to scavenge and trap free radicals that damage biomolecules [3]. Some people believe that eventually we will have a recommended minimum daily requirement for these dietary antioxidants. Much remains to be learned, before it occurs [4].

2. Materials and Methods

2.1 Materials

There is little information about nutritional and bioactive components in frozen fruits. Quickly frozen fruits for commercial use are: raspberries, strawberries and sour cherries. Examined fruits were produced by Ljubljanske mlekarne - Ljubljana, Slovenia, and were obtained from the green market.

2.2 Total anthocyanins determination

This parameter was determined by use of three spectrophotometric method (method described in European Pharmacopeia, pH differential method and bisulfate method) [5].

2.2.1 Determination of total anthocyanins in fruits extracts by the pH differential method AOAC 2005.02

The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5 (Figure 1). The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds.

Anthocyanins were extracted from 2 g frozen fruit with 50% ethanol. Two dilutions of the sample (0.5 mL fruit extract) were prepared, one with 0.025 M potassium chloride buffer, pH 1.0, and the other with 0.4 M sodium acetate buffer, pH 4.5. Dilutions were equilibrated for
15 min. and measure the absorbance at 520 nm and 700 nm. The content of the total anthocyanins was expressed as mg of cyanidin-3-glucoside equivalents per 100 g of frozen fruit [6].

2.2.2. Determination of total anthocyanins in fruits extracts by the bisulphite bleaching method

Indices for anthocyanin degradation of an aqueous extract, juice, or wine can be derived from a few absorbance readings of a sample that has been treated with sodium bisulphite. Anthocyanin pigments will combine with bisulphite to form colorless sulfonic acid adduct (Figure 2).

Polymerized colored anthocyanin-tannin complexes are resistant to bleaching by bisulphite, whereas the bleaching reaction of monomeric anthocyanins will rapidly complete. The absorbance at 420 nm of the bisulphite-treated sample serves as an index for browning. Color density is defined as the sum of absorbances at the λ vis-max and at 420 nm.

The ratio between polymerized color and color density is used to determine the percentage of the color contributed by polymerized material. The ratio between monomeric and total anthocyanin can be used to determine the degradation index.

Fruit anthocyanins were extracted from frozen samples (2 g) using 0.1 % HCl (by volume, 2 mL) in 96% ethanol and 2 % aqueous HCl (by volume, 40 mL). The mixture was centrifuged at 5,000 rpm for 15 minutes. The obtained supernatant was used for the determination of total anthocyanins. The content of total anthocyanins was measured as follows: extract (10 mL) was put into two test tubes, then 15% sodium bisulphite (4 mL) was added to one tube and dd H₂O (4 mL) to the other. After 15 minutes of incubation at room temperature the absorbance was measured with the UV – VIS spectrophotometer (Carry 50 Scan, Varian) using 1 cm path length disposable cells at 520 nm.

Figure 3. Predominant structural forms of anthocyanins present at different pH levels.

Figure 4. Formation of colorless anthocyanin-sulfonic acid adducts [6]
2.2.3 Determination of total anthocyanins in fruits extracts by the European Pharmacopeia

Anthocyanins were extracted from 2 g of frozen fruit with methanol. After a period of 30 min. of incubation at room temperature centrifugation was done. Dilutions were equilibrated for 15 min. and measure the absorbance at 528 nm.

The content of anthocyanins was expressed as mg of cyanidin-3-glucoside equivalents per 100 g of frozen fruit.

2.3 Total acidity determination

Total acidity (TA) was measured according to the AOAC method 942.15 expressed in g/L citric acid (Figure 5). pH value was measured electrometrically with a pH meter (Sartorius PB11 Germany), (Figure 6), or more precisely, strawberry and raspberry pH was determined via citric acid, and sour cherries via maleic acid determination.

3. Results and Discussion

For all three methods the total anthocyanins content was calculate using cyanidin -3-glucoside coefficients (molar extinction coefficient $\varepsilon = 26,900$ L/cm x mol and molecular weight MW = 449.2 g/mol). The content of total anthocyanins was expressed as mg of cyanidin -3-glucoside equivalents per 100 g of fresh mass of edible part of fruits (Table 1).

The total content of anthocyanins in the fruit depends on: the variety, type, ripening process, the degree of maturity, production process and geographical location.

Table 1. Total content of anthocyanins in frozen fruits

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Method A mg/100g</th>
<th>Method B mg/100g</th>
<th>Method C mg/100g</th>
</tr>
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<tbody>
<tr>
<td>Raspberries</td>
<td>94.65</td>
<td>85.60</td>
<td>74.10</td>
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<td>94.23</td>
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<td>Strawberries</td>
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<td></td>
<td>36.04</td>
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<td>Mean</td>
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<td>27.53</td>
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<tr>
<td>Sour cherries</td>
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<td>51.33</td>
<td>48.07</td>
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<td>Mean</td>
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</table>
4. Conclusions

- In our study three different spectrophotometric methods were applied for determination of total anthocyanins in frozen fruit, raspberries, strawberries and sour cherries. Results obtained from investigation show that antocyanins are the most abundant in raspberries, following sour cherries and then strawberries.

- Total acidity in 100 g of frozen fruit have values from 13.2 mg to 16.7 mg acid. The pH values are approximately 4.

- Freezing on low temperatures not only allows to prolong the period of fruit consumption, but also it allows to preserve its nutritional quality, which can be seen from the results we obtained from our research.

- Anthocyanin pigment content has a critical role in the color quality of many fresh and processed fruits and vegetables. Thus, accurate measurement of anthocyanins, along with their degradation indices, is very useful to food technologists and horticulturists in assessing the quality of raw and processed foods. Since many natural food colorants are anthocyanin derived (e.g., grape-skin extract, red-cabbage extract, purple-carrot extract), the same measurements can be used to assess the color quality of these food ingredients.

- In addition, there is intense interest in the anthocyanin content in food and nutraceuticals due to possible health benefits such as reduction of coronary heart disease, antioxidant and anticancer activities.

5. References


