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EVOLUTION OF BIOACTIVE COMPOUNDS IN FRUIT JUICES DURING PRESERVATION BY REFRIGERATION

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The study aims at determining the degree of decomposition of bioactive compounds from fruit juices during preservation by refrigeration. The fruit juices contain a number of antioxidants, vitamins, mineral salts that have a beneficial effect on the health of consumers, both from the point of view of prevention and cure. The following parameters were assayed: anthocyanins, vitamin C, tanning substances. There were used juices obtained from fruits of elder, beetroot, red grapes. The juices were preserved at +4 deg.C in absence of air. Preservation of fruit juices at the refrigeration temperature is shown to conserve anthocyans and tannins, but less vitamin C, which in the case of studied fruit juices falls with 91.43%. The anthocyans presented a decrease of 33.12%; therefore juice registered a high stability of color after storage period. The tannin content was lower by 37.62% after the storage period.

INTRODUCTION

The red fruit juices contain many bioactive compounds such as vitamins and phenolic substance, which have antioxidant properties. They show significant human health benefits. Foods are designed to provide appropriate nutrients for the metabolism, but also for the pleasure of eating them.[1] According to the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE) functional foods beneficially influence one or more functions of the body, improve health effects and/or reduce the risk of disease. Studies within the European Union research projects have highlighted a number of health benefits associated with consumption of functional food constantly (immune function, gastrointestinal health, mental health, elderly health), reducing the risk of chronic disease, obesity.[2-4]

Because of the high content of the bioactive compounds, the studied fruits (elder, beetroot, red grapes) are considered functional foods, at the boundary between food and medicine.[2] The literature mentions the presence of the bioactive compounds, such as ascorbic acid, tocoferols, polifenols, polyphenolic antioxidants, flavones, carotenoids, and minerals.[5-7]

The fruit of elderberry should be black, very ripe, because unripe fruits contain toxic glycosides: cyanogenic glycosides, sulfur steroidal glycoalkaloids, anthracene.[8,9] Dry ripe fruit contain rutoside isoquercetin, anthocyanins, amino acids, organic acids (citric, malic), tannins, sugars (up 9%), vitamin C (twice richer than lemons and oranges), B vitamins, provitamin A. They are also rich in minerals: potassium (300 mg%), calcium (35 mg%) and moderate in magnesium (9 mg%).[3,10] The skin contains sambucin, a substance similar to

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alkaloids.[11-13] The fruit of elderberry is depurative and laxative. The elderberry juice has antineuralgic properties especially in the form with low alcoholic extract, action confirmed by experimental research and clinical applications. It is used both in rheumatism, diseases of the airways, in trigeminal neuralgia and sciatica.[1,5] Red beet (*Beta vulgaris*) presents a higher nutritive value, but also valuable therapeutic properties. With a view to improve the nutritive qualities and to obtain a maximum sanitary security, the red beet juice can be fermented lactic.[14,15] Beets contain an abundance of minerals, vitamins, flavonoids, natural sugar, and a good quality and quantity of amino acids.[16,17] Betacyanins is the pigment that gives red-purple colour to beet and makes it have strong antioxidant properties. Beetroot contains mainly potassium and folic acid (Vitamin B9) that helps in regulating blood pressure and heart protection. It also prevents heart disease – beetroot fibers were shown to have the ability to lower cholesterol.[17,18]

In a study on rats with hypertension, a diet based on beet fiber resulted in a reduction of serum cholesterol and lowering triglycerides and in a significant increase in HDL cholesterol. Recent studies showed that red beet juice has effect on cancer cells being similar to a cytostatic agent doxorubicin (Adriamycin). The results suggest that betanin, the major component of betacyanin, may play an important role in the cytotoxicity exhibited by the red beetroot extract. The effect is lower, but its association with doxorubicin is recommended in order to decrease negative side effects.[19] The other studies have shown the role of antidiabetic for red beet. The bioactive molecules found in red beet, as well as folic acid, iron, carotenoids, betalains, flavonoids, vitamin B complex, fibers, minerals, pectin, ascorbic acid, in combination with *Gymnema silvestra* cause a decrease of blood glucose from 232 mg/dL to 173 mg/dL after 6-8 months of ingestion in the form of capsules, 150 mg/dL after 16 to 18 months and to 152 mg/dL after 20 to 24 months.[20-22]

Grapes, compared to other products, blood alkaline, increase the oxidative processes in the body, stimulate the activity of the stomach, endocrine glands, increase resistance to infections, strengthen the nervous system. The most important biocompounds are: tannins, phenols, flavonoids, bioflovanoids, vitamins, minerals, antioxidants and polyphenols, such as quercetin, resveratrol and catechins.[15,23,24] All these raise the level of HDL ("good" cholesterol) and reduce LDL ("bad" cholesterol). Antioxidant capacity of polyphenols was determined by a new method that is based on coupling the production of O_2^{-}• radicals, generated by the XA-XOD enzymatic system, with the amperometric detection of H_2O_2, by using a low applied potential biosensor involving Os-wired HRP.[25] The family of European *Vitis vinifera* vines is characterized by anthocyanins, which have only one molecule of glucose, while non-vinifera vines such as *Vitis labrusca* grapes and American hybrids have anthocyanins with two molecules of glucose. The tannins decrease with increasing the keeping period, their determination is a test for assessing the wines age.[26-28]

Wines have the same functional importance, but their use must be done with moderation, because recent research suggest that alcohol consumption causes a significant change in the lipid profile and this may be associated with some pathological changes observed in the kidney after ethanol consumption.[29]

RESULTS AND DISCUSSION

In Table 1 was presented the content of the vitamin C initially and after the preservation period. Initially the elderberry fruit juice had the highest content of vitamin C, 45.6mg/100g, but after three months of preservation the vitamin C content decreases with 92.87%. The same trend was seen also in the case of the other juices, the average decrease in vitamin C content being of 91.43%. "Lidia" hybrid grapes juice had a low content of vitamin C 3.9 mg/100g.

Table 1

The decreasing of the vitamin C content during preservation at the refrigeration temperature, mg/100g

Time (months)	Beetroot juice	Elderberry juice	Merlot grape juice	"Lidia" hybrid grapes juice
0	10.07	45.6	9.1	3.9
1	7.15	22.75	6.5	1.95
2	3.25	14.3	1.3	1.3
3	1.17	3.25	0.65	0.325

Table 2

The evolution of the anthocyanins content during preservation, g/L

Time (months)	Beetroot juice	Elderberry juice	Merlot grape juice	"Lidia" hybrid grapes juice
0	0.54	0.840	0.450	0.3750
1	0.48	0.765	0.412	0.3225
2	0.45	0.705	0.330	0.2700
3	0.36	0.675	0.277	0.2250

Table 3

The dinamic of tanins content, g/L

Time (months)	Beetroot juice	Elderberry juice	Merlot grape juice	"Lidia" hybrid grapes juice
0	3.059	3.22	2.89	1.449
1	2.737	3.059	2.73	1.127
2	2.254	2.89	2.57	0.960
3	1.610	2.41	1.93	0.800

Because research has demonstrated the benefits of the consumption of anthocyanins on human health, it is important to maintain anthocyanins in bigger quantity in the juices studied. In this respect the values obtained are shown in Table 2.

The elderberry juice had the highest content of anthocyanins, 0.84g/L. Elder fruits and grapes contain anthocyanins in the skin, but the ratio of the skin and fruit pulp is lower in elder, therefore the obtained juice will be more concentrated in anthocyanins than Merlot grape juice that had 0.45 g/L and "Lidia" grape juice with 0.375 g/L. The anthocyanins had the highest remanence in elderberry juice, 80.35%. The most unstable from this point of view was "Lydia" grape juice, 60% of anthocyanins in the juice being preserved after three months of storage.

Tannins are polymers of flavan and depending on the reactions to which they participate they can be hydrolyzed and unhydrolyzed. The highest content of tannins was in the elder juice, 3.22g/L, followed by beetroot juice, Merlot grape juice and hybrid grape juice, according to Table 3. During the storage the soluble hydrolysable tannins are decomposed into glucose and phenolic acids: gallic, digallic and ellagic. After 3 months of keeping at 4 degrees Celsius, anthocyanins content decreased by 49.74% for noble grape juice, with 47.36% for the juice of beet, with 44.78% for the hybrids grape juice and with 25.15% for the elderberry juice. Because in the elderberry juice tannins were best preserved, there were inhibiting enzymes in juice that contributed to the preservation of the product.

EXPERIMENTAL

Materials

There were used the juices obtained from elder fruits (*Sambucus nigra*), beetroot (*Beta vulgaris* L.), red grapes. There were used the fruits of elder from *Sambucus nigra* variety collected in the month of August, harvested at maturity (black in color). They were processed according to the technology of obtaining clear juice. For analyses was used beetroot from *Beta vulgaris rubra* variety. Beets were subjected to preliminary cleaning operations, crushing and extraction, after which raw juice was obtained.

Grape juice was obtained by processing Merlot noble grapes and Lydia hybrid variety (*Capsunica roza*). Merlot variety is part of the family of European *Vitis vinifera* vines. Grapes of *Capsunica roza* variety belong to the family of vitis non-vinifera, *Vitis labrusca*. The grapes of this variety are medium sized, with big, oval and pink berry, with thick skin. The fresh fruit juice has been bottled in tightly closed glass bottles. For each stage of determination there was a separate recipient. The preservation has been made at refrigerator at a temperature of 4 deg. Celsius.

Methods

The following parameters were assayed: anthocyanins, vitamin C, tanning substances. The anthocyanins were extracted with alcohol solution and was measured the spectrophotometrically absorption at specific wavelengths, next was calculated the intensity of coloring. The characteristic wavelength, known as X max, has been determined by a spectrophotometrical method whose principle consists in the graphic representation of the extinct variation according with the wavelength, within a range of 400-700 nm, obtaining the so-called spectral or absorption curves. In this case λ max = 535 nm.

Determination of vitamin C was performed by iodometric method. The principle of the method consists of extraction with HCl 2% of the vitamin, followed by titration with potassium iodate in the presence of potassium iodide and starch until blue coloration.

The tanning substances were determined spectrophotometrically at wavelengths of 405nm in 1 cm cuvette, using distilled water as a control. The method is based on the selective absorption in the ultraviolet tanning substances.

CONCLUSIONS

The highest content of tannins, by 3.22g/L, was determined in elderberry juice, followed by red beetroot juice – 3.059 g/L, respectively by Merlot grape juice. The mean of the tannin content decrease in the analysed samples was by 41.75%. Because the tannins act as antioxidants and preservatives, the juices colour and flavour were maintained in the following order: Elderberry juice, red beetroot juice, Merlot grape juice, "Lidia" hybrid grapes juice.

Referring to anthocyanins, these were retrieved in the analysed juices in a higher proportion than the tannins, their content decrease being by 33.12%. The average of remanence was by 66.88%, only the colour intensity being substantially changed during the period of the study.

The ascorbic acid content decreased in all the analysed samples, reported to the initial values, the average value of the decrease being by 91.44%. It has been found that the preservation of the fruit juices through refrigeration does not significantly change the content of the bioactive compounds, excepting the vitamin C. Thus, the refrigeration can be applied in order to preserve the fruit juices in terms of anthocyanins and tannins content and less of vitamin C.

The method is recommended both for fruit juices preservation for their direct consumption and for fruits preservation, with the aim to extract the anthocyanins that can be used instead of synthetic food dyes.

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