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SUGAR-FREE CONCENTRATED PRODUCTS FROM *ARONIA MELANOCARPA* FRUITS, APPLES AND CARROTS WITH ANTIOXIDANT POTENTIAL FOR PEOPLES WITH DIABETES AND OBESITY

Luminița CATANĂ[1], Monica CATANĂ[1], Anda-Grațiela BURNETE[1], Adrian Constantin ASĂNICĂ[2], Monica-Alexandra LAZĂR[1], Florica CONSTANTINESCU[1], Răzvan Ionuț TEODORESCU[3], Nastasia BELC[1]

[1]National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 DinuVintila, District 2, 021102, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, Bucharest, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Land Reclamation and Environmental Engineering, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: mcatana1965@gmail.com

Abstract

Diabetes mellitus is the most prevalent endocrine disease in the world and is likely to be the major epidemic in human history. The main causes of death due to complications of diabetes are cardiovascular disease and stroke. Due to their complex biochemical composition, especially their high polyphenol content and antioxidant potential, Aronia melanocarpa fruits have many therapeutic properties, such as the ability to fight hyperglycemia-induced oxidative stress and the macrovascular complications of diabetes, including cardiovascular disease. The aim of this work was to obtain of sugar-free concentrated products from Aronia melanocarpa fruits, apples, and carrots with high nutritional value and antioxidant capacity for the prevention and diet therapy of diabetes. These products were analyzed and characterized qualitatively (sensory, physico-chemical and microbiological). The sugar-free concentrated products are characterized by their vitamin C content (24.55-27.28 mg/100g), total polyphenol content (235.48-252.65 mg GAE/ 100 g), potassium (159.65-193.45 mg/100 g), calcium (39.35-55.67 mg/100 g), magnesium (18.85-29.74 mg/100 g), iron (1.85-2.23 mg/100 g) and zinc content (1.16-1.35 mg/100 g). Also, the sugar-free concentrated products have antioxidant capacity. These products are destined for diabetics, obesity and peoples who want to maintain their weight.

Key words: *Aronia melanocarpa, apple, carrot, diabetes, sugar-free products.*

INTRODUCTION

Diabetes mellitus (DM) is among the most prevalent chronic diseases in the world, as stated by the International Diabetes Federation (IDF). In 2017, there were approximately 451 million diagnosed patients and 5 million deaths worldwide were related to DM (Cho et al., 2018). DM refer to a group of metabolic disorders characterized by chronic hyperglycemia, resulting from impaired insulin production by pancreatic β-cells and/or insulin resistance by peripheral tissues (Goyal & Jialal, 2018). Genetic, environmental and lifestyle (diet and exercise) factors are among the different causes that can promote or prevent DM development. Moreover, oxidative stress has been strongly associated with DM pathogenesis and the progression of its comorbidities (Fernández-Ochoa et al., 2020).

In diabetes, diet has a therapeutic role being adapted to the metabolic needs of each individual.

The development of sugar-free products, but with appropriate sensory characteristics, without changing the glycemic balance of diabetic patients, is of real interest, both for engineers from food industry and for nutritionists (Catană et al., 2019).

Black chokeberry (*Aronia melnocarpa*) is a source of many bioactive compounds with a wide spectrum of health-promoting properties. Fresh fruits are rarely consumed due to their astringent taste, but they are used in the food industry for the production of juices, nectars, syrups, jams, wines, tinctures, fruit desserts, jellies, fruit teas and dietary supplements. Polyphenols are components that determine the high bioactivity of chokeberries, some of the richest sources of polyphenols, which include anthocyanins, proanthocyanidins, flavonols and phenolic acids (Sidor and Gramza-Michałowska, 2020). *Aronia melanocarpa* fruits have antioxidant, anti-inflammatory and anti-aging effects because their fruits have very high content of anthocyanins. Jeon et al. (2018) studied the effect of *Aronia melanocarpa* fruits on type 1 diabetes *in vivo* and *in vitro.*

The authors reported that these fruits decrease the blood glucose level when it's high and also has the protection effect of pancreas β cell. This study confirms the anti-diabetic effects of *Aronia* berry and is of real interest to increase its utilization in diet therapy and the treatment of diabetes. Mu et al. (2020) studied the influence of *Aronia melanocarpa* berry extract on hepatic insulin resistance in type 2 diabetes mellitus rats. The results of this study indicated that extract from the berries of *Aronia melanocarpa,* exert hypoglycemic effects in animal models of diabetes. So, it reduced levels of blood glucose, improved glucose tolerance, increased hepatic glycogen content, and regulated activity of enzymes involved in glucose metabolism.

The aim of this work was to obtain sugar-free concentrated products from *Aronia melanocarpa* fruits, apples, and carrots with high nutritional value and antioxidant capacity for the prevention and diet therapy of diabetes.

MATERIALS AND METHODS

Samples

The fruits of *Aronia melanocarpa*, which were provided by University of Agronomic Sciences and Veterinary Medicine of Bucharest (Faculty of Horticulture). *Jonathan* variety apples and carrots, were purchased from the market.

As sweetening agent, a mixture of *Stevia rebaudiana* extract and erythritol, allowed in diabetic diet, was used.

The *Sea Buckthorn* juice was used as acidifier, but also for the vitamin C and β-Carotene fortification of sugar-free jams. Low methoxyl pectin, was used to gel the sugar-free jams (the total soluble solids = 15-45°Brix).

The achieved products were sensory, physico-chemical and microbiologically analysed (Figures 1 and 2).

Figure 1. Sugar-free concentrated product from *Aronia* and apples (left: general appearance; right: product appearance)

Figure 2. Sugar-free concentrated product from *Aronia,* apples and carrots (left: general appearance; right: product appearance)

Methods

Statistical Analysis

The sugar-free concentrated products were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis

Sensory analysis (appearance, colour, taste and flavor) was performed by descriptive method and by *"Comparison method with unitary score scales"* method. Sensory quality of the fortified product was established based on the total average score by comparison with a scale from 0 to 20 points (18.1-20 - "very good"; 15.1-18 - "good"; 11.1-15 - "satisfactory"; 7.1-11 - "unsatisfactory"; 0-7 **-** "inadequate").

Measurement of the colour parameters (L^*, a^* and b^*) of samples was performed using a CM-5 colorimeter (Konica Minolta, Japan) and SpectraMagic NX software.

The textural properties (firmness, cohesiveness, adhesiveness and gumminess) of the sugar-free concentrated products were performed using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) and Bluehill 3.13 software.

Physico-chemical analysis
Total soluble solids were performed with refractometer. Chemical composition was determined by AOAC Methods: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content).
The samples were mineralized by calcination. The minerals potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) were determined by atomic absorption spectrophotometry. Phosphorus was determined by spectrophotometric method (McKie & Mccleary, 2016).
The crude fibre content of the samples was determined by gravimetric method (Fibretherm-Gerhardt equipment).
Determination of vitamins C and B content was performed by HPLC-HRMS (Asănică et al., 2019).
Determination of β-carotene content was performed by liquid chromatography with diode array detection (Catană et al., 2020).

Total polyphenol content
Total polyphenol content was performed by Folin-Ciocalteau method according to Horszwald and Andlauer (2011) using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 755 nm. The calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The polyphenol extraction solvent was a mixture of methanol and water (methanol: water = 1: 1). Total polyphenol content was expressed as mg of Gallic Acid Equivalents (GAE) per 100 g product.

Antioxidant capacity
Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging assay, according to Horszwald and Andlauer (2011),) using UV-VIS Jasco V 550 spectrophotometer, at a wavelenght λ = 517 nm. The calibration curve of Trolox achieved in the concentration range 0-0.4375 mmol/L. Antioxidant capacity was expressed as μmol Trolox Equivalents per g product.

Microbiological analysis
For the microbiological analysis of canned food SR 8924:1995 was used. Canned food was analized for *Enterobacteriaceae* presence according to SR EN ISO 21528-2:2017 method, *Escherichia coli* according to SR ISO 16649-2:2007 method and *Salmonella* according with method decribed in SR EN ISO 6579-1:2017.

RESULTS AND DISCUSSIONS

Sensory analysis
Sensory analysis revealed that the sugar-free concentrated products from *Aronia* and apples are well-gelled, it has a reddish-brown color in which purple *Aronia* fruits are distinguished. Also, Sensory analysis revealed that the sugar-free concentrated product from *Aronia,* apples and carrots are well-gelled, has a reddish-brown color with purple Aronia fruits and orange carrot noodles.
Colour instrumental analysis (Figure 3) revealed that sugar-free concentrated product from *Aronia* and apples is the darkest, recording the minimum value of luminance (L* = 10.97), and that obtained from *Aronia,* apples and carrots is the lightest (L* = 12.18).

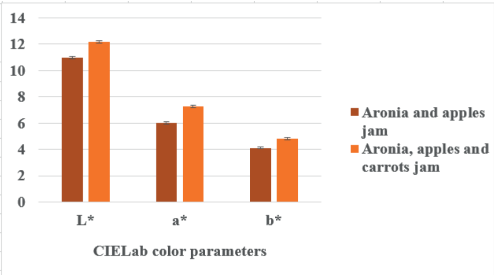

Figure 3. Colour parameters of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

Also, the maximum positive value of parameters a* (red colour coordinate) and b* (yellow colour coordinate) were recorded for the sugar-free concentrated products from *Aronia,* apples and carrots (a* = 7.27; b*= 4.8). Following the sensory evaluation, using "*Comparison method with unitary score scale*", the sugar-free concentrated products obtained "*very good*" qualifying, recording the following scores: 19.68 points (*Aronia* and apples jam) and 19.84 points (*Aronia,* apples and carrots jam) (Figure 4).

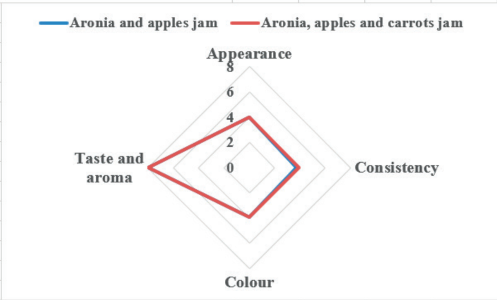

Figure 4. Sensory evaluation of the sugar-free concentrated products from *Aronia,* apples and carrots

The textural properties (firmness, cohesiveness, adhesiveness, gumminess) of the sugar-free concentrated products are presented (as mean± standard deviation) in Table 1 and the compression curves in Figures 5 and 6.

Table1. Texture properties of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

Sugar-free concentrated product	Firmness (N)	Cohesiveness	Adhesiveness (N)	Gumminess (N)
Aronia and apples jam	0.21±0.01	0.70±0.02	3.29±0.10	0.16±0.02
Aronia, apples and carrots jam	0.32±0.02	0.83±0.06	5.15±0.65	0.27±0.03

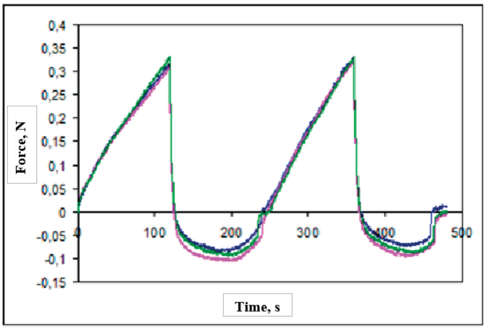

Figure 5. Compression curves of the sugar-free concentrated product from *Aronia* and apples

The sugar-free concentrated product from *Aronia,* apples and carrots had the highest values of firmness (0.32N), cohesiveness (0.83), adhesiveness (5.15) and gumminess (0.27), compared to that of the product achieved from *Aronia* and apples.

The firmness of the products (pectic gel formation) was ensured by the native pectic substances from *Aronia*, apples and carrots, by the optimal acidity (0.75-0.77 g citric acid/ 100 g) and by the addition of low methoxyl pectin.

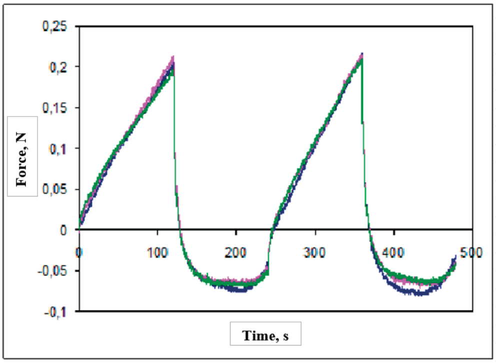

Figure 6. Compression curves of the sugar-free concentrated product from *Aronia,* apples and carrots

To obtain the compression curve (Force = f (time)) for each sample, three determinations were made. The curves obtained had a high accuracy (they were almos overlapping).

Physico-chemical analysis

Composition of the sugar-free concentrated product from *Aronia,* apples and carrots is presented (as mean ± standard deviation) in Table 2. Physico-chemical parameters of the *Aronia,* apples and carrots jam have higher values compared to those of the *Aronia* and apples jam.

Table 2. Physico-chemical composition of the sugar-free concentrated products from *Aronia,* apples and carrots

Parameters	Sugar-free concentrated product	
	Aronia and apples jam	*Aronia,* apples and carrots jam
Total soluble solids (°Brix)	30.8±0.02	32.6±0.02
Acidity (g citric acid/100g)	0.75±0.01	0.77±0.01
Total sugar (%)	6.08±0.02	6.87±0.03
Protein (%)	1.21±0.01	1.39±0.01
Fat (%)	0.49±0.006	0.54±0.008
Crude fibers (%)	2.82±0.05	3.23±0.06
Ash (%)	1.25±0.012	1.44±0.014
Pectic substances (% calcium pectate)	3.45±0.10	3.90±0.11

The total soluble solids (TSS) content of sugar-free concentrated products in Aronia, apples and carrots was higher than that reported by Sutwal et al. (2019) in the case of low-calorie apple jam (natural sweetener Stevia - concentration 0.6%; TSS = 23.30°Brix), but

comparable to that reported by Catană et al. (2019) in the case of sugar-free jams from artichoke tubers and apples (sweeteners *Stevia rebaudiana* and erythritol; TSS = 31.5-32.5°Brix).
Acidity content of the sugar-free concentrated products from *Aronia,* apples and carrots is higher than those reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples (Acidity = 0.67-0.70%) and by Sutwal et al. (2019) in the case of low-calorie apple jam (Acidity = 0.57%). The total sugar content of the sugar-free concentrated products from *Aronia,* apples and carrots achieved in this experimental study is comparable to that reported by Catană et al. (2019) in case of sugar-free jams from artichoke tubers and apples from Jerusalem artichoke tubers and apples. Also, this chemical parameter is about 2.6 times lower than that obtained by Sutwal et al. (2019) in the case of low-calorie apple jam (Total sugar = 16.64%).
The low sugar content of the products is ensured by the raw materials (*Aronia* and apples, *Aronia,* apples and carrots, respectively) and the natural sweetener used.
Proteins content of the sugar-free concentrated products achieved in this study recorded lower values about 2 times in the case of proteins compared to those obtained by Perumpuli et al. (2019) for hypoglucidic Beetroot (*Beta vulgaris* L.) jam (Protein = 2.6 ± 0.03%). Also, crude fibres content is comparable to that reported by this authors (Crude fibres = 3.5 ± 0.04%).
Also, the sugar-free concentrated products achieved in this study, stand out through their content in pectic substances (*Aronia* and apples jam = 3.45% calcium pectate; *Aronia,* apples and carrots jam = 3.90% calcium pectate).
Pectins have many beneficial effects on the body: immunomodulatory, anti-inflammatory, antitussive, anticoagulant, anticancer, gastro-protective, antidiabetic, hypolipidemic, antio-xidant effects. The effect of pectin is due to its good gelation, non-toxicity, high stability, biocompatibility, bioavailability (Zaitseva et al., 2020).
The sugar-free concentrated products from *Aronia,* apples and carrots have a high content of minerals (K, Ca, Mg, P, Zn and Fe) (Figures 7 and 8). Mineral content of the sugar-free concentrated product from *Aronia,* apples and carrots is higher than that recorded for the product obtained from *Aronia* and apples.
Sugar-free jams were noted for their potassium content (159.65 ± 13.25 mg/100 g for *Aronia* and apples jam; 193.45 ± 16.06 mg/100 g for *Aronia,* apples and carrots jam), calcium content (39.35 ± 4.06 mg/100 g for *Aronia* and apples jam; 55.67 ± 5.74 mg/100 g for *Aronia,* apples and carrots jam) and their magnesium content 18.85 ± 2.99 mg/100 g for *Aronia* and apples jam; 29.74 ± 4.71 mg/100 g for *Aronia,* apples and carrots jam).

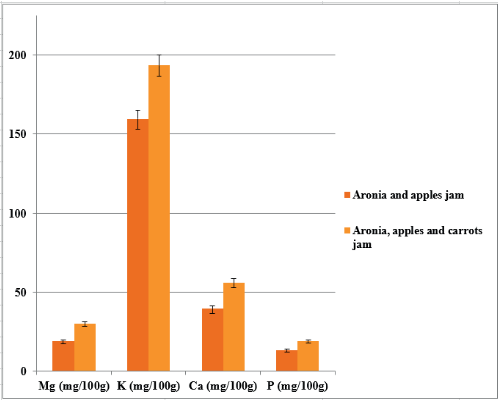

Figure 7. Mineral content (K, Ca, Mg and P) of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

The potassium content of the sugar-free concentrated products is 1.6-1.9 times smaller than that reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples and 7-8 times higher than that obtained by Perumpuli et al. (2019) for hypoglucidic Beetroot (*Beta vulgaris* L.) jam (22.378 ± 0.232 mg/100 g).
These values of the potassium content can be explained by the high potassium content of raw materials used in experiments (*Aronia melanocarpa* fruits, apples and carrots) compared to those of Beetroot (*Beta vulgaris* L.).
Adequate potassium intake can reduce blood pressure and the risk of kidney stones and osteoporosis (Freitas, 2017).
The Dietary Guidelines Advisory Committee (DGAC, 2010) recommended a potassium intake, ideally up to 4700 mg per day for adults.

The sugar-free jams recorded an iron content of 1.85 ± 0.056 mg/100 g for *Aronia* and apples jam and 2.23 ± 0.067 mg/100 g for *Aronia,* apples and carrots, respectively. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour et al., 2014).
The zinc content of the products was lower than their iron content.

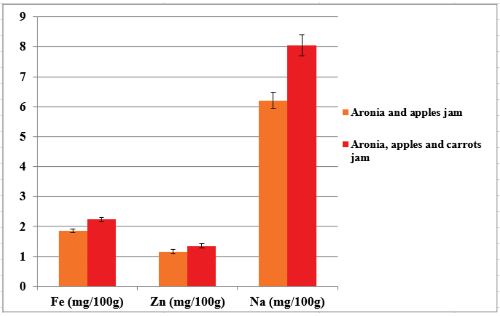

Figure 8. Mineral content (Fe, Zn, and Na) of the sugar-free concentrated products from *Aronia,* apples and carrots (the data are presented as mean ± standard deviation)

The sodium content of the sugar-free concentrated products was low (6.21 ± 0.268 mg/100 g for *Aronia* and apples jam and 8.05 ± 0.347 mg/100 g for *Aronia,* apples and carrots, respectively). Zn is an important element of the immune system. Also, Bashandy et al. (2016) showed that the protective effect of zinc can be attributed to its antioxidant and anti-inflammatory properties.

Bioactive compounds content
Sugar-free concentrated products are sources of bioactive compounds: total polyphenols, vitamin C and β-Carotene (Table 3).
The total polyphenol content of products is 1.9-2.2 times higher than that reported by Catană et al. (2019) in case of sugar-free jams from Jerusalem artichoke tubers and apples.

Many studies showed the role of phenolic compounds in the reduction of cardiovascular diseases risk factors. Thus, phenolic compounds may be considered natural inhibitors of platelet aggregation, contributing to reducing the individual risk of developing cardiovascular diseases which causes thrombosis (Lutz et al., 2019).
Also, the vitamin C content of these products is 3.5-3.9 times higher than that obtained by Sutwal et al. (2019) for sugar-free apple jam (Vitamin C = 6.90 mg/100 g). These differences can be explained by fortifying the product with *Sea Buckthorn* juice and also by the fact that for the concentration of these products, short cooking times (3-4 minutes) and long diffusion times (30-40 minutes) were applied.
Vitamin C is an important antioxidant. It functions as a redox buffer which can reduce, and thereby neutralize, reactive oxygen species.

Table 3. Bioactive compounds content of the sugar-free concentrated products from *Aronia,* apples and carrots

Bioactive compounds	Sugar-free concentrated product	
	Aronia and apples jam	*Aronia,* apples and carrots jam
Total polyphenols (mg GAE/100g)	235.48±5.42	252.65±5.81
Vitamin C (mg/100g)	27.28±0.68	24.55±0.61
Vitamin B5(mg/kg)	1.853±0.019	1.924±0.019
Vitamin B6 (mg/kg)	0.683±0.006	0.695±0.006
β-Carotene (mg/100g)	0.125±0.002	2.432±0.039

It is a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis, and regenerating other antioxidants; which also regulates cell division and growth, is involved in signal transduction, and has roles in several physiological processes, such as immune stimulation, synthesis of collagen, hormones, neurotransmitters, and iron absorption, has also roles in detoxifying the body of heavy metals (Pehlivan, 2017). Also, vitamin C stimulates the production and activation of immune cells, so perhaps supplementation could be used to improve the immunity of the cancer patients (Gorkom et al., 2019).
The vitamin B content (vitamin B5 and vitamin B6) of sugar-free concentrated products is low.
The *Aronia,* apples and carrots jam it stands out by β-Carotene content (2.432 ± 0.039 mg/ 100 g).
Carotenoids play an important role in disease prevention caused by oxidative stress, due to their antioxidant activity (Queiroz et al., 2020).
International studies show that the beneficial effect of carotenoids on the human body is due to their ability to neutralize free radicals. (Goyal, & Jialal, 2015).

Antioxidant capacity

Due to their content in antioxidants (total polyphenols, vitamin C, β-Carotene etc.) sugar-free concentrated products from *Aronia,* apples and carrots have antioxidant capacity.

The antioxidant capacity of *Aronia* and apples jam is 27.4 μmol Trolox Equivalents/g and 31.12 μmol Trolox Equivalents/g, for *Aronia,* apples and carrots jam, respectively.

Antioxidant capacity of sugar-free jams from *Aronia,* apples and carrots is similar with that reported by Abolila et al. (2015) for hypoglucidic orange jams sweetened with various sweeteners (fructose, stevioside and sucralose), which varied in the next range: 17.63-39.15 μmol Trolox Equivalents/g

Microbiological analysis

Results of the microbiological analysis of the sugar-free concentrated products from *Aronia,* apples and carrots are presented in Table 4.

Table 4. Microbiological analysis of the sugar-free concentrated products from *Aronia,* apples and carrots

Microbiological indicators	Sugar-free concentrated product	
	***Aronia* and apples jam**	***Aronia*, apples and carrots jam**
Yeast and mold (CFU/g)	Absent	Absent
Salmonella (CFU/25 g)	Absent	Absent
Enterobacteriaceae (CFU/g)	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10
Total viable count (CFU/g)	< 10	< 10

After incubation at 37°C for 14 days, the products did not show any changes of outer part of the jars and/or leakage of content, odour and/or other changes caused by a microbial activity. Microbiological analysis shown that the products are within the legislation into force. At the same time, on the basis of microbiological and sensory analysis, the shelf life of the products was established (18 months). Sugar-free products should be stored in a dark, dry, cool places at a maximum temperature of 25°C. After opening, the products should be stored at 2-8°C and consumed within 12 days.

CONCLUSIONS

The sugar-free concentrated products from *Aronia,* apples and carrots achieved in this experimental study using as sweeteners *Stevia rebaudiana* and erythritol had superior sensory characteristics (appearance, colour, flavor, taste and texture) like the jams obtained with sugar.

The sugar-free products obtained within this study are important sources of bioactive compounds (polyphenols, vitamin C, β-Carotene) and minerals (K, P, Fe, Mg, Ca).

Also, these products are noted by total polyphenol content (235.48 ± 5.42 mg GAE/100 g for *Aronia* and apples jam; 252.65 ± 5.81 mg GAE/100 g for *Aronia,* apples and carrots jam), vitamin C content (27.28 ± 0.68 mg/100 g for *Aronia* and apples jam; 24.55 ± 0.61 mg/100 g for *Aronia,* apples and carrots jam) and β-Carotene (2.432 ± 0.039 mg/100 g for *Aronia,* apples and carrots jam).

The sugar-free concentrated products from *Aronia,* apples and carrots have antioxidant capacity and their consumption in a healthy diet can prevent diseases caused by oxidative stress.

The sugar-free concentrated products from *Aronia,* apples and carrots presented in this paper can be successfully introduced in the diet of diabetics, obese and people who want to maintain their weight.

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