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Aronia Extracts in the Production of Confectionery Masses

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Abstract: The article examines the opportunity to use extracts and Aronia melanocarpa (Michx.) Elliot fruit powders in the production of sugar confectionery for the substitution of synthetic dyes. In the technology of manufacturing confectionery masses, synthetic dyes are used that can cause various allergic reactions, as well as hyperactivity syndrome and lack of concentration in children. The composition of hydroalcoholic extracts was analyzed, and the metabolites of polyphenols, individual anthocyanins and organic acids were quantified. Antioxidant capacity and CIELab chromatic parameters were tested. The technology for manufacturing confectionary masses with extract and powder of aronia was developed. The sensory profile, physicochemical and microbiological quality parameters, antioxidant activity and color characteristics of the confectionary masses with the extract and powder of aronia addition were determined on the 1st and 50th day from the production date. The evolution of DPPH antioxidant activity of confectionery masses during storage was measured in vitro, in the conditions of gastric digestion. The results showed that Aronia melanocarpa (Michx.) Elliot extract is rich in polyphenols, flavonoids and tannins, the main organic acids being represented by malic, citric, acetic and ascorbic acid. During the 50th storage day, the antioxidant activity was higher in confectionery masses containing aronia compared to the control. The sensory and microbiological testing of confectionary masses demonstrated that the combination of extract and aronia powder ensures the optimal shelf life and organoleptic scores. It was demonstrated that during the storage of confectionery masses with aronia, the physicochemical indicators of quality were in accordance with the regulated admissible values. Positive effects of aronia were observed on confectionery masses' color saturation. These results underline the opportunity to use aronia extract and/or powder in confectionery industry to replace synthetic dyes and obtain products with enhanced functionality.

Keywords: Aronia melanocarpa (Michx.) Elliot; biologically active compounds; antioxidant activity; color parameters; sugar products; quality characteristics

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1. Introduction

Aronia (Aronia melanocarpa (Michx.) Elliot) is widely distributed in eastern, southern, and central parts of Europe, being grown on an industrial scale [1]. Aronia fruits are used in the manufacture of juices, purees, jams, jellies and wine [2]. This is due to the high content of polyphenols, with considerable antioxidant activity and a remarkable coloring potential [3,4].

Among the polyphenols present in aronia, quercetin is the strongest antioxidant among the monomer phenolic compounds, followed by cyanidol-glucoside and chlorogenic acid [5]. Anthocyanins, flavonols and hydroxycinnamic acids contribute about 59.4% of the total antioxidant activity of aronia without assuming the possible synergism/antagonism between individual antioxidants [6]. About 40% of antioxidant activity can be attributed to

proanthocyanidins, the main antioxidants of aronia fruit [7]. Bushmeleva et al., showed that anthocyanins in aronia fruit show a pronounced reduction and antiradical activity, which exceeds the corresponding indices of other polyphenols and vitamin C [8]. The aronia fruits, due to their high biologically active compounds (BAC) content, have a wide range of pharmacological effects, such as pronounced antioxidant activity and medicinal and therapeutic benefits: gastroprotective, hepatoprotective, antiproliferative and anti-inflammatory [9]. Aronia can help prevent chronic diseases, metabolic disorders, diabetes and cardiovascular disease [10]. The health benefit of aronia fruits against inflammation in RAW 264.7 cells has been shown [11]. These fruits show antimicrobial activity against the pathogenic bacteria *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [12].

Confectionery masses are used as a filling for candies, biscuits and other pastries. To assign the color red, synthetic dyes such as azorubine (E122), amaranth (E123), ponceau 4R (E124), erythrosine (E127) and allura red AC (E129) are used [13], which can affect the health of consumers often causing allergic reactions and aggravating asthma [14]. Foods containing azorubine (E122) are not recommended for children because this substance causes the syndrome of hyperactivity and lack of concentration [15]. In this context, in order to replace synthetic dyes, there is an opportunity to use extracts and aronia fruit powder in the production of sugar confectionery. In addition, there is a need in the development of technology for the manufacture of sugar products with natural dyes, beneficial to the health of the human body. The application of BAC from powders and extracts of aronia fruit in the creation of sugar products for the replacement of synthetic dyes is particularly current [16,17].

Thus, the aim of this paper was to underline the possibility to use aronia extract and powder for the enrichment of confectionery masses. For this purpose, the aronia extract was characterized regarding the content of bioactive compounds, organic acids and color parameters. Then, the effects of aronia extract and powder on confectionery masses' properties on the 1st day and after 50 days of storage were evaluated by investigating the sensory profile and physicochemical and microbiological characteristics, including antioxidant activity.

2. Materials and Methods

2.1. Chemical Materials

The Folin–Ciocalteu reagent, acetonitrile, formic acid, tannic acid, trans-resveratrol and cis-resveratrol were purchased from Merck (Darmstadt, Germany); (+)-catechin (98%), (−)-epicatechin (98%), syringic acid, ferulic acid, gallic acid (98%), protocatechuic acid, para-hydroxybenzoic acid, meta-hydroxybenzoic acid, p-coumaric acid (≥98%), salicylic acid (99.9%), polydatin (≥95%), ferulic acid methyl ester, vanillic acid (97%), DPPH, ascorbic acid, citric acid (99.5%) and acetic acid (99.8%) were obtained from Sigma-Aldrich (Darmstadt, Germany; Tokyo, Japan; Shanghai, China). Sinapic acid (98%) was purchased from Alfa Aesar (Kandel, Germany). Procyanidin B1, procyanidin B2 and hyperoside were purchased from Extrasynthese (Genay, France). Cyanidin 3-glucoside chloride (≥98%), cyanidin 3-arabinozide chloride (≥95%), cyanidin 3-galactozide chloride (≥95%) and petunidin 3-glucoside chloride (≥98%) were obtained from PhytoLab, (Vestenbergsgreuth, Germany). All spectrophotometric measurements were performed on the Analytik Jena Specord 200 Plus (Jena, Germany) spectrophotometer.

2.2. Biological Material

Aronia melanocarpa (Michx.) Elliot (black chokeberry) of the variety "Nero" were harvested at the end of August, by the company "BerryMania" SRL (Cuizăuca, Republic of Moldova), which has its own plantations over 4 ha near Cuizăuca village in the Rezina district, located in the northeastern part of the Republic of Moldova (47°36′46″ latitude, 28°48′28″ longitude and altitude of 163 m above sea level). Berries were dried at a temperature of 65 ± 1 °C to a humidity of 8.3 ± 0.2%, crushed to powder with a particle size of 60 ± 10 µm and then sieved.

2.3. Extract Characterization

An amount of 2 g of aronia powder was extracted with 28 mL of 60 % ethanol under stirring at 60 rpm for 90 min at temperature of 65 °C [18]. After filtration, the polyphenol, flavonoid, tannin, anthocyanin and organic acid composition, antioxidant activity and color parameters were determined. The extract was stored in glass bottles at 4.0 ± 1.0 °C, in the dark.

2.3.1. Total Polyphenols and Flavonoids by Folin–Ciocalteu

The method described by Singleton et al. [19] was used to determine the total polyphenol content. The results were calculated from a calibration curve using gallic acid (0–500 mg/L, $y = 0.0012x + 0.0076$, $R^2 = 0.9985$) and expressed in equivalents of gallic acid per 100 g of dried weight (DW) of aronia extract (mg GAE/100 g DW). The total flavonoid content was calculated by measuring the difference between the total polyphenol content until and after the precipitation of flavonoids with formaldehyde in a strong acidic medium [20]. The results were expressed in mg GAE/100 g DW.

2.3.2. Total Tannins by Folin–Ciocalteu

The Folin–Ciocalteu reagent was used to determine the tannin content according to the method described by Waterman and Mole [21]. The results were calculated from a calibration curve using tannic acid (0–50 mg/L, $y = 0.0011x + 0.0128$, $R^2 = 0.9982$) and expressed in equivalents of tannic acid per 100 g of dried weight (DW) of aronia extract (mg TAE/100 g DW).

2.3.3. Total Anthocyanins

The total anthocyanin content was determined using method described by Giusti and Wrolstad [22]. The results were expressed in equivalents of cyanidin-3-glucoside per 100 g of dried weight (DW) of aronia extract (mg CGE/100 g DW).

2.3.4. HPLC Analysis of Polyphenols

Using the HPLC Agilent 1100 Series (Santa Clara, CA, USA) the content of individual polyphenols in the ethanolic aronia extract was determined. The gradient was optimized using trifluoroacetic acid (TFA) as an eluent acidification of 1% CH_3OH (A channel) and 50% CH_3OH (B channel) acidified to 2.15 pH with TFA. The column system was composed of a pre-column SecurityGuard ULTRA Cartridges HPLC (Torrance, CA, USA) C18 for 4.6 mm ID coupled with a Kinetex 5 µm C18 100 Å 250 × 4.6 mm column manufactured by Phenomenex at 35 °C. A run time of 90 min and an injection volume of 20 µL were used. The phases were A: H_2O:CH_3OH (99:1) and B: H_2O:CH_3OH (50:50), with a flow of 1.5 mL/min. The detection was carried out at 256, 280, 324 and 365 nm. The gradient of elution was 100% (A): for 10 min; 82% (A): 18% (B) for the next 10 min; 70% (A): 30% (B) for 10 min; 65% (A): 35% (B) for 6 min; 40% (A): 60% (B) for 15 min; 20% (A): 80% (B) for 5 min; 100% (B) for 15 min and 100% (A) for 10 min. The content of specific polyphenols was determined by comparison of retention times and peaks of the sample chromatogram with ones from the chromatogram of synthetic standards listed in Table 1. The linearity (R^2), limit of detection (LOD) and limit of quantitation (LOQ) are presented in Table S1.

Table 1. Characteristics of polyphenol standards used in HPLC analysis and their retention times.

Compound	Max Absorption (nm)	Retention Time (min)
Gallic acid	280	5.294
Protocatechuic acid	256	9.267
p-Hydroxybenzoic acid	256	13.918
Procyanidin B1	280	16.704
m-Hydroxybenzoic acid	280	17.989
Catechin	280	18.53
Vanillic acid	324	22.871
Procyanidin B2	280	23.433
Syringic acid	280	25.002
Epicatechin	280	26.836
p-Coumaric acid	324	29.695
Ferulic acid	324	36.233
Salicylic acid	280	36.995
Polydatin	280	38.234
Sinapic acid	324	38.564
Hyperoside	288	47.305
trans-Resveratrol	324	49.333
cis-Resveratrol	324	57.089
Ferulic acid methyl ester	365	57.754

2.3.5. HPLC Analysis of Anthocyanins

The content of individual anthocyanins in the ethanolic aronia extract was analyzed by direct separation by HPLC Agilent 1100 Series HPLC (Santa Clara, CA, USA), using reverse phase column with gradient elution by water/formic acid/acetonitrile with detection at 518 nm [23]. The identification of anthocyanins from samples was carried out by the comparison of UV–VIS spectra and retention times of the sample peaks with those of the standard solutions (Table 2). The R^2, LOD and LOQ are presented in Table S2.

Table 2. Anthocyanins used as standards in HPLC analysis and their retention times.

Compound	Cyanidol-3-galactozide	Cyanidol-3-arabinozide	Cyanidol-3-glucoside	Petunidol-3-glucoside
Retention Time (min)	8.862	9.805	10.593	11.034

2.3.6. Quantification of Organic Acids

The Agilent 7100 CE system (Santa Clara, CA, USA) and the method described by Cristea et al. [24] were used to quantify organic acids in extract. The total organic acid content was expressed in mg/100 g DW of aronia extract.

2.3.7. Antioxidant Activity by Reaction with DPPH Radical

The method described by Brand-Williams et al. [25] was used to measure the antiradical DPPH activity of ethanolic aronia extract. The results were expressed in mmol trolox equivalents per 100 g of dried weight (DW) of aronia extract (mmol TE/100 g DW) after the calibration curve (0–250 µmol/L, $y = 0.0029x + 0.0108$, $R^2 = 0.9985$) created using trolox as standard.

2.3.8. Color Parameters (CIELab)

WinASPECT PLUS software (Jena, Germany) and a Specord 200 Plus spectrophotometer (Jena, Germany) were used to evaluate the color parameters (CIELab). Luminosity (L*), red/green component (a*), yellow/blue component (b*), chromaticity (C*) and hue angle (H*) are presented as results. These parameters were measured following the official method [26].

2.4. Confectionery Masses Making

Samples of confectionery masses were made using aronia extract (CMAE) and the combination of extract and aronia powder (CMAEP) to determine its effects on organoleptic and physicochemical indicators, color parameters and microbiological stability of the confectionery samples. The control sample was prepared without the addition of extract or aronia powder. Sugar, molasses, cocoa butter, skimmed milk powder and 60% ethyl alcohol (v/v) were used in the making of confectionery masses. The production technology provides the preparation of sugar–molasses syrup (water consumed constitutes one third of the total quantity of sugar), with a dry-matter content of 78.0 $\pm$ 0.1%. In the syrup cooled to 64 $\pm$ 1 °C, cocoa butter and skimmed milk powder were added and mixed until a homogeneous mass was obtained. During mixing, the mass temperature was reduced to 55 $\pm$ 1°C and aronia extract 60% (v/v) was added, which changed the color and rheological properties of the confectionery mass and CMAE sample was obtained. Subsequently, the addition of aronia powder in the amount of 5% in relation to the mass of the product allowed to obtain the CMAEP sample. The confectionery masses were shaped into balls, cooled, dried at ambient temperature, packed in bags, and stored at room temperature. The control was prepared similarly to the use of 60% (v/v) ethanolic solution, without vegetable additives. The specific quantities of the manufacturing recipe were 664.0 g sugar, 143.0 g molasses, 115.0 g cocoa butter, 63.0 g skimmed milk powder and 15.0 mL ethanolic solution 60% (v/v) (control). In the case of samples with a vegetable addition, 15 mL of ethanolic solution was replaced with the same amount of aronia extract (CMAE) and 5 g of aronia powder (CMAEP) was added.

2.5. Confectionery Masses Analysis

The confectionery masses were analyzed on the 1st and 50th days from production date in order to study the parameters' evolution during storage.

2.5.1. Sensory Analysis

Standard ISO 6658:2017 [27] was followed when performing the sensory analysis of the products. Appearance, taste, odor, color, and consistency were assessed using the 5-point system by an expert panel of eleven trained food technologists. The 5-point assessment system includes the following scores: 5—very good; 4—good; 3—satisfactory; 2—poor; 1—bad; and 0—very bad.

2.5.2. Physicochemical Analysis

The moisture was assessed by using the weight loss method. The sample was heated at 105 °C to a constant weight [28]. The pH was determined by the express method using the Testo 205 pH meter (Testo Ltd., Alton, UK), used for determinations in semisolid substances in food production. The determination of water activity (a_w) was performed by the express method using the LabSwift-aw device (Novasina AG, Lachen, Switzerland).

2.5.3. Fat Content

The method for determining the fat content was used, which is based on the action of concentrated sulfuric acid and isoamyl alcohol on the released fats and the measurement of their volume in the graduated part of the butyrometer [29].

2.5.4. Reducing Substance Content

The iodometric method was used to determine the reducing substances according to AOAC method [28].

2.5.5. Microbiological Analysis

Standard ISO 4833-2:2013/COR 1:2014 [30] was followed when performing the microbiological analysis. The total viable count (TVC) of mesophilic aerobic organotrophic

bacteria was determined following incubation at temperature of 30 °C for 4–72 h using nutrient agar.

2.5.6. In Vitro Antioxidant Activity of Confectionery Masses

In vitro antioxidant activity (DPPH) was measured in the conditions of gastric digestion using the method described by Ghendov-Mosanu et al. [31]. The DPPH radical scavenging activity of the clear solution was measured at room temperature following the method of Brand-Williams et al. [25]. The results were expressed as μmol TE/100 g from a calibration curve (0–250 μmol/L) with trolox.

2.5.7. Color Parameters of Confectionery Masses

The Konica Minolta CM-3600d colorimeter and the method described by Ścibisz et al. [32] were used to measure the color parameters of confectionery masses.

2.6. Statistical Analysis

All calculations were performed using Microsoft Office Excel 2007 (Microsoft, Redmond, DC, USA). Data obtained in this study are presented as mean values ± the standard error of the mean calculated from three parallel experiments. The comparison of average values was based on the one-way analysis of variance (ANOVA) according to Tukey's test at significance level $p \leq 0.05$, using the Staturphics program Centurion XVI 16.1.17 (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results and Discussion

3.1. Characterization of Aronia Extract

This section presents the characteristics of aronia extract that are needed in order to understand the phenomena that occur when it is added to confectionery. The composition of polyphenols, anthocyanins and individual organic acids, antioxidant activity and color parameters for the aronia extract are shown in Table 3.

Table 3. Polyphenols, anthocyanins and individual organic acids, antioxidant activity and color parameters (CIELab) in aronia extract (the results are expressed as means ± standard deviations of three experiments).

Indices	Quantity
Polyphenols	
Total polyphenol content (mg gallic acid equivalents (GAE)/100 g DW)	5522 ± 125
Total flavonoid content (mg GAE/100 g DW)	5071 ± 68
Tannin content, (mg tannic acid equivalents (TAE)/100 g DW)	549.2 ± 15.6
Gallic acid (μg/100 g DW)	20.97 ± 0.63
m-Hydroxybenzoic acid (μg/100 g DW)	6.99 ± 0.43
Protocatechuic acid (μg/100 g DW)	101.08 ± 0.98
p-Hydroxybenzoic acid (μg/100 g DW)	11.29 ± 0.21
Syringic acid (μg/100 g DW)	2.69 ± 0.05
Ferulic acid (μg/100 g DW)	296.24 ± 1.21
Sinapic acid (μg/100 g DW)	4.30 ± 0.02
Catechin (μg/100 g DW)	828.49 ± 14.32
Epicatechin (μg/100 g DW)	252.69 ± 3.56
p-Coumaric acid (μg/100 g DW)	3.23 ± 0.35
Vanillic acid (μg/100 g DW)	4.84 ± 0.24
Salicylic acid (μg/100 g DW)	142.47 ± 1.92
Hyperoside (μg/100 g DW)	52.15 ± 0.68
Procyanidin B1 (μg/100 g DW)	14.52 ± 0.21
Procyanidin B2 (μg/100 g DW)	6.45 ± 0.09
Polydatin (μg/100 g DW)	68.28 ± 0.26
trans-Resveratrol (μg/100 g DW)	0.27 ± 0.04
cis-Resveratrol (μg/100 g DW)	0.59 ± 0.05
Ferulic acid methyl ester (μg/100 g DW)	79.57 ± 0.97

Table 3. *Cont.*

Indices	Quantity
Anthocyanins	
Total anthocyanin content (mg cyanidin-3-glucoside equivalents (CGE)/100 g DW)	412.0 ± 1.1
Cyanidol-3-galactozide (mg/100 g DW)	263.7 ± 1.8
Cyanidol-3-arabinozide (mg/100 g DW)	125.7 ± 1.4
Cyanidol-3-glucoside (mg/100 g DW)	11.5 ± 0.5
Petunidol-3-glucoside (mg/100 g DW)	11.1 ± 0.9
Organic acids	
Malic acid (mg/100 g DW)	4836 ± 15
Citric acid (mg/100 g DW)	362 ± 9
Ascorbic acid (mg/100 g DW)	66 ± 3
Acetic acid (mg/100 g DW)	312 ± 10
Antioxidant activity	
Antioxidant activity (DPPH) (mmol trolox equivalents (TE)/100 g DW)	191.66 ± 5.32
CIELab Chromatic Parameters	
L*	30.21 ± 0.17
a*	34.72 ± 0.05
b*	9.46 ± 0.08
C*	36.00 ± 0.06
H*,°	15.2 ± 0.3

DPPH = 2,2-diphenyl-1-picrylhydrazyl-hydrate, L*—luminosity; a*—red/green component; b*—yellow/blue component; C*—chromaticity; H*—hue angle.

3.1.1. Phenolic Content in the Aronia Extract

The results show that the aronia extract was rich in biologically active compounds. Therefore, the total polyphenol content was 5522 mg GAE/100 g DW extract; the total flavonoid and tannin content were, respectively, 5071 mg GAE/100 g DW and 549.2 mg TAE/100 g DW extract. Vinogradova et al., studied four aronia specimens and showed that the species and districts of fruit growth influenced the total content of polyphenols and flavonoids. In the case of A. melanocarpa extract, they were, respectively, 40.06 mg GAE/g and 13.16 mg QE/g [33]. Lazarova et al., studied the influence of extraction temperature on the total polyphenol content in hydroalcoholic extracts from aronia waste and found that at 73.3 °C the extraction rate of polyphenols increased 4.5 times (7700 mg GAE/L) compared to 40 °C (1711 mg GAE/L) [34]. Denev et al., studied 23 samples of aronia fruits (Nero variety) in which the total polyphenol content ranged from 1022.4 to 1795.5 mg GAE/100 g FW [35]. Bibliographic sources attest that the variety, cultivation and harvesting conditions can significantly influence the total polyphenol content, the values of which can reach up to 8000 mg/100 g DW [36]. Gralec et al., demonstrated that the ripening state of the aronia fruits influenced the flavonoid content, showing that during the ripening period it decreased from 7–11 g/100 g DW to 4 g/100 g DW [37]. According to the study by Tolic et al., in dried fruits and powders of aronia the flavonoid content varied between 867 mg GAE/100 g DW and 3317 mg GAE/100 g DW [38]. In the study by Mladin et al., it was shown that in the aronia fruits harvested in 2006–2007, the tannin content varied between 1.174 g/100 g and 1.072 g/100 g fruit [39].

Table 3 shows the polyphenols identified by the HPLC method in hydroethanolic extract from aronia fruits. The main phenolic compounds detected in aronia extract were catechin (828.49 µg/100 g DW), ferulic acid (296.24 µg/100 g DW), epicatechin (252.69 µg/100 g DW), salicylic acid (142.47 µg/100 g DW), protocatechuic (101.08 µg/100 g DW), ferulic acid methyl ester (79.57 µg/100 g DW), polydatin (68.28 µg/100 g DW), but also gallic, para- and meta-benzoic acids, procyanidin B1 and B2, etc. Tolić et al., identified and quantified polyphenolic compounds, especially gallic, caffeic, p-coumaric, ellagic, chlorogenic and flavonols acids (quercetin and kemferol) in two varieties of fresh aronia fruits (Nero and Viking variety) [5]. Ciocoiu et al., used high-performance liquid chromatogra-

phy coupled with diode array detection and electrospray ionization–mass spectrometry (HPLC/DAD/ESI-MS) and identified five phenolic compounds in the ethanolic extract of aronia fruits as follows: chlorogenic acid, kuromanin, rutin, hyperoside and quercetin [40]. Nowak et al., identified phenolic acids, especially chlorogenic, neochlorogenic and coumaric acids, and quercetin, catechin and epicatechina were detected from the flavone group [41]. Zheng and Wang identified caffeic acid and its derivatives in wild aronia [42]. Significant amounts of caffeic and ferulic acids were detected by Häkkinen et al. [43].

3.1.2. Anthocyanin Content in the Aronia Extract

Aronia fruits are characterized by an increased content of coloring pigments that can be used to replace synthetic dyes in confectionery. The data in Table 3 show that in the aronia extract, the total anthocyanin content was 412 mg CGE/100 g DW extract. Tolić et al., investigated the total anthocyanin content in different products obtained from aronia and reported that in dried fruits and in tea, this parameter varied in the range of 141 mg CGE/100 g DW–675 mg CGE/100 g DW [39]. Wathon et al., investigated the conditions for the anthocyanins extraction from aronia skin wastes and showned that the anthocyanin content varied from 3.4 to 10.0 mg/g DW [1]. Park and Hong investigated the influence of solvent type on the anthocyanin extraction from aronia fruits and demonstrated that the 50% hydroethanolic solution extracted a higher amount of anthocyanins (318.61 mg/100 g) than hot water (252.82 mg/100 g) [44].

Three different cyanidol-glucosides: 3-galactoside, 3-arabinoside, 3-glucoside and petunidol-3-glucoside were identified in the aronia extract. The amounts of cianidol-3-galactoside (263.7 mg/100 g DW) and cianidol-3-arabinoside (125.7 mg/100 g DW) were predominant, and content of cianidol-3-glucoside (11.5 mg/100 g DW) and petunidol-3-glucoside (11.1 mg/100 g DW) were lower. Jakobek et al., determined four different cyanidol-glucosides: 3-galactoside, 3-arabinoside, 3-glucoside and 3-xyloside. It was found that cyanidol-3-galactoside and cyanidol-3-arabinoside accounted for over 93% of the total anthocyanin content, and cyanidol-3-xyloside (3.6%) and cyanidol-3-glucoside (3%) were identified in relatively small quantities [45]. The same anthocyanin profile in aronia extracts was identified by Banach et al. [9]. Denev et al., published a review and found that in fruits and juice from aronia, cyanidol-3-galactoside and cyanidol-3-arabinoside were identified in large quantities, and cyanidol-3-glucoside and cyanidol-3-xyloside—in small amounts. Pelargonidol-3-arabinoside was identified only in aronia fruits [7]. Variations in the chemical structure of anthocyanins are mainly due to differences in the number of hydroxyl groups in the molecule, the methylation degree of these hydroxyl groups, the nature and number of monosaccharides [46].

3.1.3. Organic Acids in the Aronia Extract

The organic acids identified and quantified in the aronia extract are shown in Table 3. In our case, organic acids were represented by the following acids: malic (4836 mg/100 g DW), citric (362 mg/100 g DW), acetic (312 mg/100 g DW) and ascorbic (66 mg/100 g DW). Denev et al., researched 23 samples of aronia fruits cultivated in Bulgaria and quantified the organic acids which varied in the following ranges: quinic (232.4–591.0 mg/100 g fresh weight (FW)), malic (229.5–435.5 mg/100 g FW), ascorbic (37.3–91.8 mg/100 g FW) and citric (18.3–41.3 mg/100 g FW), and succinic, shikimic and oxalic acids were identified in small quantities [36]. It is obvious that the content and kind of organic acids were influenced by climatic conditions of cultivation region, degree of ripeness and the fruit variety [47,48].

3.1.4. Antioxidant Activity in the Aronia Extract

Bibliographic sources attest that polyphenolic compounds are responsible for the antioxidant activity in plant materials [49,50]. Phenolic compounds, due to their antioxidant properties, facilitate the elimination of free radicals and prevent the conversion of hydroperoxides into free radicals [51]. Antioxidant activity in the aronia extract was measured by DPPH, the value being 191.66 mmol TE/100 g DW (Table 3). For the measurement of

antioxidant activity in various aronia samples, researchers use different analytical methods and reference standards that lead to difficulty in comparing the results obtained by other authors [9,36,39,52]. In our research, the antioxidant activity in aronia extract was also determined by the photochemiluminescence test [53] and by the ABTS method [54]. However, only the results obtained by the DPPH method were presented in this article, which are the most relevant [18].

3.1.5. CIELab Chromatic Parameters

The color of the aronia extract, which is due to the presence of anthocyanins, is a very important feature when used as a natural dye in the manufacture of confectionery [15,16]. The CIELab chromatic parameters of the extract are shown in Table 3. The prevalence of red pigments (a* = 34.72) and the reduction of yellow pigments (b* = 9.46) were demonstrated in the aronia extract. The chromaticity parameter demonstrated the color intensity of the extract, the value being C* = 36.00. Due to the predominance of red tone, the hue angle H* = 15.2° was in the first trigonometric quadrant. Bibliographic sources attest that the stability of anthocyanins in extracts depends on their chemical structure and their interaction with other food components and may be influenced by pH, storage temperature, the presence metal ions, etc. [4,15].

3.2. Confectionery Masses Characterization

Table 4 presents the sensory profile, physicochemical and microbiological quality parameters, antioxidant activity and color characteristics of the confectionary masses with the addition extract (CMAE) and powder of aronia (CMAEP), compared to the control prepared without any addition. The evolution of quality indicators, i.e., sensory characteristics, moisture content, active acidity pH, water activity (a_w) and total viable count (TVC) during storage, was determined on the 1st and 50th day from the production date. The fat content and the mass fraction of the reducing substances were determined only on the first day.

Table 4. Sensory profile, physicochemical and microbiological quality indicators, antioxidant activity and color characteristics of confectionary masses with added extract and aronia powder compared to the control (the results are presented as means ± standard deviation).

Quality Indicators	Confectionary Masses					
	Control		CMAE		CMAEP	
	1st Day	50th Day	1st Day	50th Day	1st Day	50th Day
Sensory profile total score	17.31 ± 0.15 ^{a,b}	16.90 ± 0.12 ^{a}	19.71 ± 0.04 ^{c}	18.59 ± 0.08 ^{c}	24.85 ± 0.05 ^{e}	23.76 ± 0.07 ^{d,e}
Appearance	3.51 ± 0.03 ^{a}	3.49 ± 0.05 ^{a}	4.22 ± 0.02 ^{b}	4.14 ± 0.04 ^{b}	5.00 ± 0.0 ^{c}	5.00 ± 0.0 ^{c}
Taste	3.05 ± 0.04 ^{a}	3.00 ± 0.01 ^{a}	3.91 ± 0.02 ^{b}	3.83 ± 0.03 ^{b}	5.00 ± 0.0 ^{c}	4.80 ± 0.05 ^{c}
Odor	3.08 ± 0.03 ^{a}	3.02 ± 0.02 ^{a}	3.52 ± 0.01 ^{c}	3.31 ± 0.03 ^{b}	4.85 ± 0.01 ^{e}	4.40 ± 0.0 ^{d}
Color	3.61 ± 0.04 ^{b}	3.58 ± 0.03 ^{b}	3.91 ± 0.03 ^{c}	3.45 ± 0.02 ^{a}	5.00 ± 0.0 ^{e}	4.78 ± 0.05 ^{d}
Consistency	4.06 ± 0.06 ^{b,c}	3.81 ± 0.05 ^{a}	4.15 ± 0.06 ^{c}	3.86 ± 0.04 ^{a,b}	5.00 ± 0.0 ^{e}	4.78 ± 0.03 ^{d}
Moisture content (%)	6.23 ± 0.14 ^{b}	6.20 ± 0.10 ^{b}	6.21 ± 0.12 ^{b}	6.19 ± 0.10 ^{b}	4.12 ± 0.07 ^{a}	4.10 ± 0.09 ^{a}
Active acidity pH	6.12 ± 0.07 ^{e}	5.86 ± 0.08 ^{c,d}	6.02 ± 0.06 ^{d,e}	5.75 ± 0.07 ^{c}	5.30 ± 0.03 ^{b}	4.78 ± 0.05 ^{a}
Water activity (a_w) (c. u.)	0.697 ± 0.003 ^{c,d}	0.695 ± 0.002 ^{c,d}	0.695 ± 0.002 ^{c,d}	0.691 ± 0.001 ^{c}	0.672 ± 0.003 ^{a,b}	0.667 ± 0.002 ^{a}
Fat content (g/100 g)	53.2 ± 0.8 ^{c}	nd	50.6 ± 1.1 ^{b,c}	nd	43.2 ± 1.2 ^{a}	nd
Mass fraction of the reducing substances (%)	11.99 ± 0.06 ^{a}	nd	12.79 ± 0.01 ^{b}	nd	13.59 ± 0.05 ^{c}	nd
Total viable count (TVC)*(CFU/g)	8 ± 2 ^{a}	60 ± 0 ^{c}	8 ± 1 ^{a}	57 ± 3 ^{c}	5 ± 1 ^{a}	41 ± 1 ^{b}
Antioxidant activity DPPH (μm TE/100 g)	3.96 ± 0.21 ^{b}	1.61 ± 0.35 ^{a}	4.57 ± 0.39 ^{b}	1.78 ± 0.42 ^{a}	8.15 ± 0.53 ^{c}	4.66 ± 0.31 ^{b}
L*	92.05 ± 1.42 ^{c}	94.11 ± 1.86 ^{c}	76.45 ± 1.21 ^{b}	81.45 ± 1.34 ^{b}	19.74 ± 1.15 ^{a}	23.17 ± 0.90 ^{a}
a*	−0.20 ± 0.15 ^{a}	−1.08 ± 0.43 ^{a}	32.47 ± 1.15 ^{c}	25.00 ± 1.12 ^{b}	36.17 ± 1.09 ^{d}	28.14 ± 1.08 ^{b,c}
b*	10.18 ± 0.96 ^{b}	5.01 ± 0.53 ^{a}	42.36 ± 0.78 ^{d}	36.41 ± 0.59 ^{c}	5.98 ± 0.24 ^{a}	9.00 ± 0.71 ^{b}
C*	10.18 ± 1.04 ^{b}	5.12 ± 0.67 ^{a}	53.37 ± 0.85 ^{f}	44.17 ± 0.64 ^{e}	36.66 ± 0.74 ^{d}	29.54 ± 0.45 ^{c}
H* (°)	91.1 ± 0.97 ^{d}	102.2 ± 1.45 ^{d}	52.5 ± 0.86 ^{c}	55.5 ± 1.54 ^{c}	9.40 ± 0.8 ^{a}	17.74 ± 1.78 ^{b}

Different letters ($^{a–f}$) designate statistically different results ($p \leq 0.05$); nd = not determined; * = nutrient agar; CMAE—confectionery masses made using aronia extract; CMAEP—confectionery masses made using the combination of extract and aronia powder.

3.2.1. Sensory Profile of the Confectionary Masses

In order to predict the acceptance level of the product by consumers, sensory testing of confectionary masses was performed by the specialized tasting panel. Comparing the

samples of CMAEP and CMAE with the control shows that the extract and aronia powder had a positive influence on all sensory characteristics, especially on appearance, taste, color, and consistency, which were improved for both samples (CMAEP and CMAE) at every tested time. It was shown that the highest total sensory score was accumulated by CMAEP (24.85), followed by CMAE (19.71) compared to control (17.31). Therefore, the combination of extract and aronia powder had a positive influence on the sensory properties of confectionery masses. The confectionery masses with only aronia extract showed a color, smell and taste less specific to aronia, which made the total sensory score of CMAE less important than in the case of CMAEP. During the 50-day storage period, the change in sensory characteristics was insignificant and the total sensory scores were summed as follows: 16.90 (control), 18.59 (CMAE) and 23.76 (CMAEP). Thus, for confectionery masses, the combination of extract and aronia powder is optimal, while the optimal shelf life for high organoleptic scores is 50 days.

3.2.2. Influence of Aronia on the Moisture Content

The results in Table 4 show that on the 1st day the moisture content in the control and CMAE was 6.23% and 6.21%, respectively, but in the CMAEP the value of this quality indicator decreased 1.5 times and was 4.12%. This is explained by the fact that the aronia powder had a low moisture content (8.3 $\pm$ 0.2%), and the addition of the powder contributed to the stability of the confectionery masses during storage. On the 50th day of storage, the moisture of samples decreased compared to the values obtained on the 1st day. Thus, for the control it constituted 6.20%, for CMAE and CMAEP–6.19 % and 4.10%, respectively.

3.2.3. pH Variation in the Confectionery Masses

The pH values in the confectionery masses are influenced by the chemical composition of the aronia fruits, especially by the presence of organic acids (malic, citric, acetic, etc.). Thus, the pH values in the confectionery masses were as follows: 6.12 (control), 6.02 (CMAE) and 5.30 (CMAEP). During storage for 50 days, the pH values decreased in all samples: by 4.2% (control), by 4.5% (CMAE), and by 9.8% (CMAEP) (Table 4). Probably, this decrease can be influenced not only by the chemical composition of the aronia fruits, but also due to the oxidation of the lipids present in cocoa butter used in manufacturing confectionery masses [55,56].

3.2.4. Water Activity in Confectionary Masses

Water activity (a_w) indicates the stability of food during storage (Table 4). The results obtained on the 1st day present that the value of a_w varies between 0.672 and 0.697 c. u., which corresponds to data from bibliographic sources for candy [57]. The results obtained on the 50th day of storage show that the values of this index decreased, being in the range of 0.667–0.695 c. u., showing that the multiplication and survival of vegetative cells of bacteria was stopped and the samples are stable during storage.

3.2.5. Fat Content in the Confectionery Masses

The fat content in the confectionery masses was determined on the 1st day, the values being as follows: 53.2 g/100 g (control), 50.6 g/100 g (CMAE) and 43.2 g/100 g (CMAEP) (Table 4). Tanaka T. and Tanaka A. reported that the fat content of aronia fruits due to seeds was 0.14 g/100 g FW [58]. In our opinion, this fat content cannot significantly influence this indicator in the confectionery masses. The decrease in the value of this indicator in CMAEP is probably explained by the fact that the addition of 5% aronia powder to the product mass contributed to the increase in the total mass of the finished product.

3.2.6. Reducing Substance Content in the Confectionery Masses

The mass fraction of reducing substances was determined in the confectionery masses. Due to the reducing sugars (glucose and fructose) in aronia fruits [59], the values of this

quality indicator were modified as follows: 11.99% (control), 12.79% (CMAE) and 13.59 (CMAEP), which correspond to the values allowed regulated up to max. 14.0% [60]. Thus, the physicochemical indicators of the confectionery masses' quality were in accordance with the regulated allowed values [60].

3.2.7. Total Viable Count in the Confectionery Masses

The results in Table 4 show that during the storage of fortified samples, the total viable count (TVC) was reduced compared to the control. On the 50th storage day, TVC decreased 1.46 times in CMAEP compared to control. Cisowska et al., reported on the antimicrobial activity of aronia extracts on pathogenic bacteria: *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was shown that Gram-positive bacteria were more sensitive to aronia extracts compared to Gram-negative bacteria. According to the authors, such an effect was caused by anthocyanins from aronia fruits [61]. Other sources indicate that phenolic substances, especially tannins and flavonoids, are responsible for antimicrobial activity [62,63]. The antimicrobial activity of tannins is related to their ability to inactivate microbial adhesion, enzymes and proteins inside the cells of microorganisms. In the case of flavonoids, this is due to their ability to form complexes with soluble proteins of the cell walls of microorganisms [64]. In addition, the hydroalcoholic solution and the physicochemical indicators of the quality of the confectionery masses, especially the moisture content, a_w and pH, influenced the microbiological stability. All the samples obtained both on the 1st day and on the 50th storage day had TVC in accordance with the values allowed by the regulation [65].

3.2.8. DPPH Antioxidant Activity of Confectionery Masses

An important role is played by the antioxidant character of aronia powder, which is related to the fact that their chemical composition includes a series of BAC and natural antioxidants, which essentially influence the stability and antioxidant activity of the confectionery masses. Natural antioxidants have the ability to reduce the fat oxidation of food [66]. The evolution of DPPH antioxidant activity of confectionery masses during storage was measured in vitro in the conditions of gastric digestion. It was found that all samples showed antioxidant activity as follows: 3.96 µm TE/100 g (control) and 4.57 µm TE/100 g (CMAE); and for CMAEP, the value of antioxidant activity was considerably higher—8.15 µm TE/100 g. During the 50 storage day, the values of the antioxidant activity were reduced in all the investigated samples. It was shown that antioxidant activity was higher in confectionery masses containing aronia compared to the control and was more significant in CMAEP than in CMAE (Table 4).

3.2.9. CIElab Chromatic Parameters of the Confectionery Masses

The CIElab chromatic parameters of the confectionery masses during storage were evaluated. The results attest that on the 1st day, the control was characterized by the high value of the luminosity L* (92.05). The negative value of component a* (−0.20) showed the presence of green hue, and the positive value of component b* (10.18)—yellow hue, due to the color of cocoa butter and skimmed milk powder. The chromaticity C* (10.18) demonstrated the low color intensity and the presence of gray shade. In addition, the hue angle H ° (91.1) is in the second trigonometric quadrant, in which the yellow tone predominates. In the case of fortified samples, the use of aronia extract led to a decrease in L* 76.45 (CMAE), and the addition of aronia powder contributed to a sudden reduction in this value—19.74 (CMAEP). Due to the anthocyanins in aronia fruits, the color of the CMAEP darkened. In the case of a* values, in the samples with aronia, it varied between 32.47 and 36.17, demonstrating the presence of anthocyanins. The b* values were positive in the aronia samples and vary in a fairly large range. In CMAE, the b* value increased up to 36.41 due to the presence of yellow hue, but with the addition of aronia powder, this value was drastically reduced to 9.00 (CMAEP). The C* demonstrated the color intensity of the CMAE and CMAEP, being 53.37 and 36.66, respectively. The H° is in the first trigonometric

quadrant, 52.5° (CMAE) demonstrating the presence of the orange hue, and 9.4° (CMAEP), the presence of red tone.

The shelf life influenced the chromatic parameters of the confectionery masses. On the 50th storage day, the L* value increased in all the samples studied: by 2.2% (PM); by 6.5% (CMAE) and by 17.4% (CMAEP). The a* values reduced to all the samples investigated. For PM, the a* component was negative (−1.08) due to the presence of green tone, and for the other samples the values were 25.00 (CMAE) and 28.14 (CMAEP), demonstrating the reduction in red tone compared to the initial values. The b* values were positive for all tested samples, but decreased, showing a decrease in yellow pigments. Furthermore, the C* values decreased due to the reduced color intensity of the tested samples. The H ° values attest that in all the researched samples the color hue was lighter, but they remained in the same trigonometric quadrants described above (Table 4). Several authors have noted that the color intensity of confectionery masses with aronia, which was determined by the content of monomeric anthocyanins, decreased under the influence of temperature and storage time [67].

The extract in combination with aronia powder can be successfully used in the technology of making confectionery masses as natural dyes, helping to increase the biological value of sugary products. In addition, this fact allows to expand the assortment of candies and fillings.

4. Conclusions

The Aronia melanocarpa (Michx.) Elliot extract was rich in BAC such as polyphenols, flavonoids, and tannin. The main phenolic compounds detected in aronia extract were catechin, ferulic acid, epicatechin, salicylic acid, protocatechuic, ferulic acid methyl ester, polydatin, but also gallic, para- and meta-benzoic acids, procyanidin B1 and B2. The amounts of cianidol-3-galactoside and cianidol-3-arabinoside were predominant. The organic acids were represented by the malic, citric, acetic and ascorbic acids. The CIELab chromatic parameters of the aronia extract demonstrated a prevalence of red pigments and a reduction in yellow pigments with a high coloring intensity.

Sensory testing of confectionary masses performed by the specialized tasting panel shows that the extract and aronia powder had a positive influence on all sensory characteristics, especially on appearance, taste, color, and consistency. During the 50-day storage period, the change in sensory characteristics was insignificant. The physicochemical indicators of the confectionery masses' quality were in accordance with the regulated allowed values. Water activity (a_w) varies slightly during storage. Together with the reduction in the pH value, this indicates that the multiplication and survival of the vegetative cells of the bacteria has been stopped and the samples are stable during storage. During the storage of fortified samples, the total viable count (TVC) was reduced compared to the control. The evolution of the DPPH antioxidant activity of confectionery masses during storage, measured in vitro in the conditions of gastric digestion, showed a significant increase in relation to the control. The analysis of chromatic parameters showed a positive influence of vegetable powders on the color saturation of confectionery masses, which demonstrates that the extract and aronia powder can be used successfully in the technology of making confectionery masses as natural dyes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12157664/s1, Table S1: Linearity (R2), Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the Analysis of Polyphenolic Compounds; Table S2: Linearity (R2), Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the Analysis of Anthocyanin Compounds.

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