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Nutritional and Physico-Chemical Characteristics of Innovative Bars Enriched with *Aronia melanocarpa* By-Product Powder

Bogdan Constantin Bratosin [1,2], Gheorghe-Adrian Martău [1,2], Călina Ciont [1,2], Floricuța Ranga [1], Elemér Simon [1,2], Katalin Szabo [1,2], Sorina Darjan [3], Bernadette-Emőke Teleky [1,2] and Dan Cristian Vodnar [2,*]

[1] Department of Food Science, University of Agricultural Science and Veterinary Medicine, Calea Mănăstur 3–5, 400372 Cluj-Napoca, Romania; bogdan-constantin.bratosin@usamvcluj.ro (B.C.B.); adrian.martau@usamvcluj.ro (G.-A.M.); calina.ciont@usamvcluj.ro (C.C.); floricutza_ro@yahoo.com (F.R.); simon.elemer@usamvcluj.ro (E.S.); katalin.szabo@usamvcluj.ro (K.S.); bernadette.teleky@usamvcluj.ro (B.-E.T.)
[2] Institute of Life Sciences, University of Agricultural Science and Veterinary Medicine, Calea Mănăstur 3–5, 400372 Cluj-Napoca, Romania
[3] Department of Environmental and Plant Protection, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3–5, 400372 Cluj-Napoca, Romania; sorina.dirjan@usamvcluj.ro
* Correspondence: dan.vodnar@usamvcluj.ro; Tel.: +40-747-341-881

Abstract: In a quest to meet the rising demand for nutrient-rich products, this study delves into the realm of innovative bars enriched with *Aronia melanocarpa* by-product powder. By repurposing waste material from fruit pressing, the research unveils a sustainable approach to enhancing the health profile of food products. Two variants of bars were used: one as a control and the other enriched with freeze-dried aronia by-product powder. Both bars had similar nutrient content, containing approximately 10% fibre, 12.20% protein, 20.51% fat, and 429–430 kcal calories. The investigation showcases a remarkable 61% increase in antioxidant activity in bars enriched with freeze-dried aronia powder compared to traditional bars. In vitro digestion tests demonstrated enhanced nutrient release in aronia powder-enriched bars. Moreover, a 70-day storage analysis demonstrated the microbiological stability of the bars, which is essential for their commercial viability (final total viable cell count 4.41 $\log_{10}$ CFU/g). Also, total fungi-yeast and moulds increased to 4.17 ± 0.131 $\log_{10}$ CFU/g for aronia bars and to 3.91 ± 0.107 $\log_{10}$ CFU/g for control bars after 70 days of storage. This collaborative research effort not only sheds light on the nutritional and physico–chemical characteristics of the innovative bars but also propels the development of functional food products with heightened antioxidant content and bioactive compounds.

Keywords: bioactive compounds; antioxidant content; in vitro digestion; freeze-drying; functional food development; microbiological stability; sustainable practices

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1. Introduction

Aronia melanocarpa, known as black chokeberry, offers fruits rich in bioactive compounds with remarkable nutritional and biological values [1–3]. Among these compounds, polyphenols, emphasizing anthocyanins, are the dominant group, providing astringent taste and distinctive colour [4,5]. The benefits of these fruits are supported by multiple studies that have identified various positive effects on health. For example, anthocyanins have positively influenced circulation and heart health, stimulating insulin secretion and improving retinal health [6–8].

According to Jurikova et al. [9], fruits of *A. melanocarpa* L. are an essential source of antioxidants, especially polyphenols such as phenolic acids and proanthocyanidins. Due to the high content of such bioactive compounds, these fruits bring significant health benefits, including solid antioxidant protection and potential therapeutic effects on gastrointestinal,

hepatic, and inflammatory conditions. Their effects on blood pressure, lipid profile, and glucose levels are essential in preventing metabolic disorders, diabetes, and cardiovascular diseases. The popularity of these fruits is on the rise due to their association with multiple health benefits, from antioxidant, antibacterial, and antiviral properties to potential anticancer and anti-inflammatory actions [9].

In 2022, the aronia berries market reached a value of approximately USD 720 million globally. It is anticipated to expand to about USD 1342 million by 2030, showcasing a compound annual growth rate of approximately 7.3% from 2023 to 2030 [10,11]. Research conducted by Cvetanović et al. [12] and Kokotkiewicz et al. [13] has emphasized the nutritional benefits of products derived from *A. melanocarpa*. The fruits undergo dehydration, grinding, and transformation into powders, making them a favourable choice for inclusion in various food processing applications, such as nutrition bars, instant teas, fruit yoghurts, desserts, and dietary supplements [12,13].

Furthermore, by-products from processing, such as pomace, have been recognised as having significant nutritional value, containing polyphenols, anthocyanins, fibres, pectin compounds, minerals, and vitamins [14,15]. In Europe, 3% of the total food waste is comprised of by-products from the juicing industry, which accounts for a significant portion of food waste [16]. These by-products, which could constitute up to 20–80% of the whole fruit, are significant for the environment [17]. Therefore, due to their high antioxidant potential, these residual products can contribute to health and prevent diseases caused by oxidative stress, such as cancer, osteoporosis, cardiovascular diseases, and neurodegenerative conditions [18].

This research aims to conduct a comprehensive analysis of the benefits associated with the by-products of *A. melanocarpa*. By focusing on the potential of these by-products, the study investigates their utilization in the food industry to develop healthy and nutritionally rich foods. The primary objective is to explore the nutritional contribution and functional properties of *A. melanocarpa* by-products, elucidating their role in enhancing the health profile of food products. The study delves into various applications within the food industry, including the development of innovative bars, emphasizing the valuable role of these by-products as sources of antioxidants and other bioactive compounds.

2. Materials and Methods

2.1. Materials

Ingredients such as cashew butter, honey, coconut flakes, and oat flakes were purchased online from Dr. Green, a natural food store in Cluj-Napoca (drgreen.ro, accessed on 14 November 2023). *A. melanocarpa* pomace was bought from Romanian farmers and, until further processing, was kept at −18 °C in the freezer. It was then subjected to freeze-drying at −55 °C. Pepsin from porcine gastric mucosa (Art. No. P6887), pancreatin from porcine pancreas (Art. No. P7545), and bovine bile extract (Art. No. B8631) were acquired from Sigma–Aldrich (Taufkirchen, Germany).

The grinding of dried semi-finished products was carried out using a Retsch mill. The functional ingredients obtained (powders) were packaged in glass containers, sealed tightly, protected with an aluminum foil against light, and stored in dry and cool spaces (up to a maximum temperature of 20 °C) until biochemical analysis. Figure 1 shows the *A. melanocarpa* pomace powders obtained through freeze-drying.

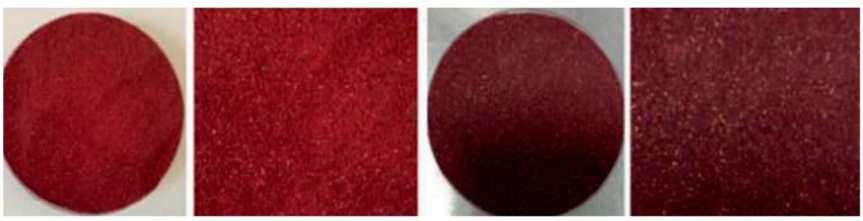

Figure 1. *Aronia melanocarpa* pomace powder (freeze-dried at −55 °C).

2.2. Bar Formulation

First, cashew butter and honey were heated in an oven at 60 °C for 3 min. Then, oat flakes and coconut flakes were added. Subsequently, the mixture was reheated and manually homogenized by continuous stirring. For the aronia-containing bar, after homogenizing the content, 3% aronia powder was added to half of it and mixed until a homogeneous paste was obtained (Table 1). Finally, both were weighed and shaped. The prepared bars were then allowed to cool at room temperature for subsequent analysis or use.

Table 1. Raw ingredients in cereal-based formulations and cereal bars with aronia by-product powder (g/100 g).

Ingredients	Control Bar	Bar with By-Product
Cashew butter	38.22%	38.22%
Honey bees	35.72%	35.72%
Oat flakes	21.06%	18.06%
Coconut flakes	5%	5%
Aronia by-product	-	3%

The influence of the storage period on microbiological analysis was also monitored over 70 days, and the bars formed were vacuum packaged (Peach Vacuum Sealer, model PH320, Schindellegi, Switzerland) individually at a quantity of 75 g/bar and stored in a dark place at room temperature.

2.3. Rheological Analyses

Dynamic rheological measurements of the protein bars were conducted using an Anton Paar MCR 72 rheometer (Anton Paar, Graz, Austria) equipped with a Peltier plate system (P-PTD 200/Air) and a temperature controller (set at T = 4 °C and room temperature). The rheometer had a smooth parallel plate with a diameter of 50 mm (PP-50-67300). Initially, 3 g of the sample was added to the center of the lower plate of the Peltier system, with a 1.5 mm gap between the plates, and allowed to rest for 5 min [19,20].

After providing the sample, any excess was removed, and silicon oil was added to prevent drying. Oscillatory frequency sweep tests were then performed at angular frequencies ranging from 0.628 to 628 rad/s to determine the dynamic storage modulus (elastic) (G', Pa) and loss modulus (viscous) (G'', Pa). G' and G'' represent the material's ability to store elastic deformation energy and the viscous portion of the material, respectively.

The results from three experiments, each with replicates, were presented as the mean value ± standard deviation (SD), where n = 3. With Graph Prism Version 8.0.1 was performed statistical analysis (GraphPad Software Inc., San Diego, CA, USA).

2.4. In Vitro Digestion

In vitro static digestion of the *A. melanocarpa* powder-enriched bar was conducted to evaluate nutrient release. Based on the detailed protocol described by Brodkorb and colleagues [21], the method involves sequential oral, gastric, and intestinal digestion steps, with parameters such as electrolytes, enzymes, bile, pH, dilution, and digestion time set based on physiological data.

The samples, including the cereal bar used as a control, a cereal bar enriched with *A. melanocarpa* freeze-dried powder, and the freeze-dried *A. melanocarpa* powder, underwent duplicate in vitro digestion in three steps to mimic the conditions in the mouth, stomach, and small intestine. Due to the absence of starch in the matrix, the oral phase omitted amylase. The samples were diluted with water and mixed with simulated oral fluid (SOF) at a 1:1 weight/weight ratio to achieve the desired consistency, creating a bolus for swallowing with a paste-like texture. SOF was composed of electrolyte solutions, including KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2{\cdot}6H_2O$, $(NH_4)_2CO_3$, as well as $CaCl_2(H_2O)_2$ and water. Additionally, 10 mL of simulated gastric fluid, consisting of electrolyte solutions such as KCl, KH_2PO_4, $NaHCO_3$, $MgCl_2{\cdot}6H_2O$, $(NH_4)_2CO_3$, were mixed with the oral

bolus, along with a rabbit gastric extract (RGE15 Lipolytech, Marseille, France) containing 60 U/mL lipase and 2000 U/mL pepsin in the final digestion mixture and water. The mixture's pH was adjusted to 3 using HCl (3 M), and for 120 min, the samples were incubated at 37 °C while rotating on an orbital shaker (45 rpm).

The samples were mixed with simulated intestinal fluid (SIF) for the intestinal phase to achieve a final 1:1 (v/v) ratio. The SIF contained electrolyte solutions such as KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2{\cdot}6H_2O$, as well as $CaCl_2(H_2O)_2$, water, pancreatic enzymes (100 U/mL in the final digestion mixture), and a bile extract solution (10 mM in total digest). The samples' pH was adjusted to 7 using NaOH (1 M), and the mixture was homogenized on an orbital shaker (45 rpm) and incubated for 120 min at 37 °C. After completing the digestion process, the samples were centrifuged at 8000 rpm at room temperature, and 1 mL of the supernatant was filtered for further analysis using high-performance liquid chromatography (HPLC) from Agilent Technologies, Santa Clara, CA, USA.

2.5. Antioxidant Activity

The antioxidant activity of the extracts, including the aronia by-product, freeze-dried powder, the prototype cereal bar, and the aronia powder-enriched bar, was assessed before and after being freeze-dried, as well as after in vitro digestion. The samples were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capacity technique. To determine the antioxidant responses of the samples, 35 µL of previously methanolic-extracted samples were mixed in triplicate with 250 µL of a methanolic DPPH solution. The reaction solution was incubated in the dark at room temperature for 30 min before measuring the absorbance at 515 nm using a multi-mode plate reader (BioTek, Winooski, VT, USA). The three experiments with replicates (n = 3) are expressed as the mean value ± standard deviation (SD). The results were presented as micromole Trolox equivalents (µmol TE) per 100 g sample [22].

2.6. HPLC–DAD–ESI–MS Identification of the Phenolic Composition

2.6.1. Extraction of Phenolic Compounds

An amount of 1 g of the aronia by-product sample and 0.5 g of aronia by-product after freeze-drying were extracted with 10 mL of methanol acidified with 1% HCl by vortexing for 1 min in a vortexer (Heidolph MR-Hei-Standard, Schwabach, German), sonicating for 15 min in an ultrasonic bath (Elmasonic E15H, Elma, Singen, Germany) and centrifuging at 10,000 rpm for 10 min at 24 °C in an Eppendorf AG 5804 centrifuge (Eppendorf, Leipzig, Germany). These operations were repeated until the sample was decolourized entirely. Additionally, 20 µL was injected into the HPLC system from the methanolic extract that had been previously filtered through a 0.45 µm Chromafil Xtra nylon filter [23]. The experiments were made in triplicate (n = 3).

2.6.2. High-Performance Liquid Chromatography (HPLC DAD-ESI$^+$) Conditions

An Agilent 1200 HPLC system equipped with an autosampler, a quaternary pump, solvent degasser, diode array UV–Vis detector (DAD), and single-quadrupole mass detector (MS) Agilent model 6110 (Agilent Technologies, Santa Clara, CA, USA) was used to identify and quantify phenolic compounds. Compound separation was performed on a Kinetex XB C18 column, dimensions 4.6 × 150 mm, with 5 µm particles (Phenomenex, Torrance, USA), using the following gradient mobile phases: (A) water +0.1% acetic acid and (B) acetonitrile +0.1% acetic acid over 30 min, at a temperature of 25 °C, with a flow rate of 0.5 mL/min. Gradient (% B): 0 min, 5% B; 0–2 min, 5% B; 2–18 min, 5–40% B; 18–20 min, 40–90% B; 20–24 min, 90% B; 24–25 min, 90–5% B; 25–30 min, 5% B. Spectral values were recorded in the range of 200–600 nm for all peaks. Chromatograms were recorded at wavelengths λ = 280, 340, and 520 nm. Various phenolic compounds were identified by comparing the retention times, UV visible and mass spectra of unknown peaks with reference standards [24]. For MS, the positive ESI ionization mode was used for a full scan. The working conditions were as follows: Temperature 35 °C, nitrogen flow rate 7 L/min, and m/z 120–1200, with

a capillary voltage of 3000 V. Data acquisition and result interpretation were performed using Agilent ChemStation software (Rev B.02.01–SR2 [260], Palo Alto, CA, USA) [25].

2.7. Nutritional Properties

The nutritional properties of the bars were obtained using AACC (2000) methods (AACC, 2000) [26]. Moisture content (AACC 44-15.02, 2000), fat (AACC 30-25.01, 2000), fibre and ash (AACC 08-01.01, 2000) were estimated using standard analytical methods. The protein content determination was carried out by converting nitrogen to protein using the Kjeldahl method, employing a factor of 5.7 (AACC 46-11.02, 2000).

Equation (1) was used to calculate the total carbohydrate content (%).

$$\text{Total carbohydrate (\%)} = 100 - [\text{moisture (\%)} + \text{ash (\%)} + \text{fiber (\%)} + \text{protein (\%)} + \text{fat (\%)}] \quad (1)$$

The caloric value was estimated according to Equation (2):

$$\text{Kcal} = (\text{Carbohydrates} \times 4.0) + (\text{Protein} \times 4.0) + (\text{Fat} \times 9.0) \quad (2)$$

2.8. Influence of Storage Period on Microbiological Analysis

Over 70 days, the total viable cells, fungi-yeast and moulds were counted and monitored. For microbiological analysis, 3 g of the bar were mixed in 27 mL sterile saline solution (0.85% NaCl), and serial dilution was made. The last 2 dilutions were taken into account for plate making. The analysis was performed in triplicate ($n = 3$). The result was displayed in logarithmic values of colony-forming units per gram of the sample ($\log_{10}$ CFU/g).

2.8.1. Total Viable Cell Count

The method used for total viable cell count (TVC) was the pour plate method (1 mL of the serial dilution was poured into the plate, over which 15 mL agar medium was added; the medium was homogenized along with the sample), and the media was nutrient agar (NA). NA (peptone 5 g/L; sodium chloride 5 g/L; HM peptone B# 1.5 g/L; yeast extract 1.5 g/L; agar 15 g/L) with a final pH of 7.4 ± 0.2 (25 °C) has been used as a general medium for cultivating less fastidious microorganisms that are present in samples (ex., *Escherichia* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Yersinia* spp.). Incubation was at 30 °C for 3 days [27].

2.8.2. Total Fungi-Yeast and Moulds

The method used for total fungi-yeast and moulds (TFYM) was the spreading method (0.1 mL of the dilution was put on the agar plate and spread with the Drigalski spatula), and Dichloran Rose-Bengal Chloramphenicol agar (DRBC) was used as a media to identify numerous fungi-yeasts and moulds. DRBC (peptone 5 g/L; dextrose (glucose) 10 g/L; potassium dihydrogen phosphate 1 g/L; magnesium sulphate 0.5 g/L; Rose Bengal 0.025 g/L; chloramphenicol 0.1 g/L; dichloran 0.002 g/L; agar 15 g/L) with a final pH of 5.6 ± 0.2 (25 °C) is recommended for selective isolation of yeasts and moulds, especially in food samples (ex., *Candida* spp., *Saccharomyces* spp., *Aspergillus* spp., *Mucor* spp.). Incubation was set at 25 °C for 5 days [27].

The TVC and TFYM were calculated using the formula specified below [27]:

$$\text{TVC AND TFYM} = \frac{\sum c}{V \times (n_1 + 0.1 \times n_2) \times d} \text{ (CFU/g)}$$

where: $\sum c$—sum of colonies per plate
V—Inoculum volume (mL)
n_1—Number of counted plates for the 1st dilution
n_2—Number of counted plates for the 2nd dilution
d—First dilution to be counted.

2.9. Statistical Analysest

Every measurement was performed in triplicate, and the findings are presented as the mean value (±SD, n = 3). Statistical analysis was performed using Graph Prism Version 8.0.1 (GraphPad Software Inc., San Diego, CA, USA) with a one-way ANOVA test coupled with Tukey's multiple comparisons test. Significance was established at a 5% confidence level for variations in means.

3. Results and Discussions

3.1. Rheological Analyses

In the context of our study, the application of dynamic rheological measurements on nutritional bars prior to manufacturing holds significant importance for several reasons. Dynamic rheology, which involves the study of a material's flow and deformation behaviour under various conditions, provides crucial insights into the structural and textural characteristics of food products. Here, we discuss the relevance of applying dynamic rheological measurements in the pre-manufacturing phase of aronia-enriched bars in comparison to control bars. To select the best composition variant for the bars, the measurements were performed on all batches at room temperature and 4 °C (Figure 2).

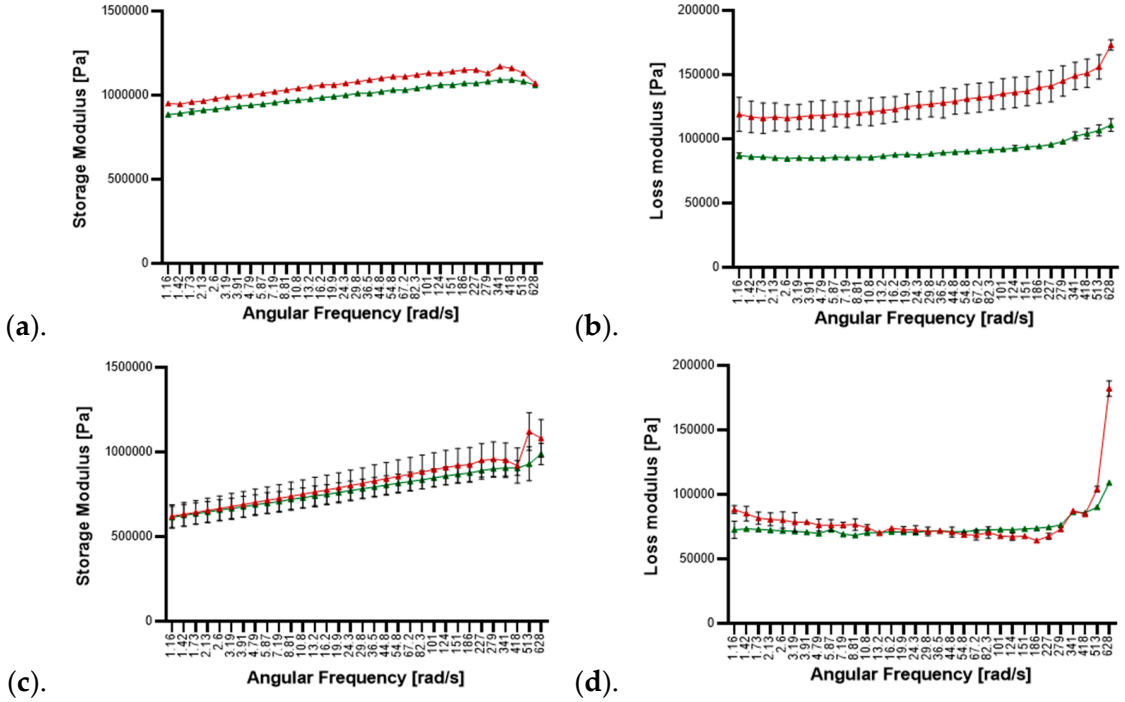

Figure 2. Properties related to the flow and deformation behaviour of the bars before and after enrichment with aronia powder at room temperature (**a**,**b**) and at 4 °C (**c**,**d**), where red is the control bar and green colour is supplemented with the aronia by-product bar.

Oat bars enriched with aronia benefit from oat starch, which is known for having distinct characteristics like small granule size, a well-developed granule surface, elevated lipid content, and variable proportions of amylose and amylopectin. This oat starch is increasingly recognized for its versatile applications in both food and non-food industries, where its pasting and rheological properties play a crucial role in maintaining product quality [28]. The incorporation of aronia powder exerted discernible effects on the dynamic rheological properties of the bars. Notably, a reduction in moduli was observed in the enriched bars, and this reduction was more pronounced compared to the batch devoid of aronia. Within each batch, the storage modulus (G′) consistently surpassed the loss modulus (G″), indicating a predominantly solid behaviour in the bars without the addition of aronia powder, as reported in previous studies on the production of functional foods [28].

The figures in Figure 2a,c provide visual representations of the G′ curves obtained from the conducted measurements. In stress measurement experiments, G″ values typically exhibit constant plateau levels at low strain values. G′ values in this plateau region, known as the linear viscoelastic (LVE) region, measure the material's elasticity under minimal deformation and represent its structural resistance or mechanical stiffness at rest [29]. Specifically, structural modifications were observed in the bars without aronia powder selected as the model variant. However, these modifications were notably improved after the addition of aronia powder, leading to the selection of variant aronia by-product bars. The observed alterations in rheological properties align with the structural changes induced by the inclusion of aronia powder, signifying the potential impact of this additive on the textural characteristics of the bars. This suggests a meaningful enhancement in the overall structural integrity and mechanical properties of the bars with the incorporation of aronia powder.

3.2. Antioxidant Activity and Bioactive Compounds

The results presented in Table 2 reveal a noteworthy contrast in antioxidant activity between freeze-dried aronia and aronia pomace. Freeze-dried aronia exhibited significantly higher antioxidant activity, registering a value of 10,621.67 ± 0.28 µM Trolox/100 g of dry weight, marking a substantial 61% increase. In comparison, aronia pomace recorded a value of 4140.1 ± 0.18 µM Trolox/100 g of fresh weight. Notably, findings from a study by Lazăr et al. [30] reported a Trolox equivalent of 2.95 µmol/g for aronia powder, highlighting its elevated antioxidant activity. This significant difference in antioxidant activity can be attributed to the freeze-drying process, known for its ability to concentrate antioxidant compounds in aronia. Therefore, freeze-dried aronia can be considered a notably potent source of antioxidants compared to aronia pomace [30]. The concentration of antioxidant compounds in freeze-dried aronia indicates its potential to be a more concentrated and bioactive source, affirming its superiority over aronia pomace in terms of antioxidant potential, along with the impact of processing techniques on the antioxidant content of aronia-derived products.

Table 2. Antioxidant activity of extracts before and after freeze-drying.

Freeze-dried aronia	10,621.67 ± 0.28 µM Trolox/100 g dry weight
Fresh aronia	4140.1 ± 0.18 µM Trolox/100 g fresh weight

Table 3 highlights a substantial contrast in total phenolic compounds between aronia pomace and freeze-dried aronia. Aronia pomace exhibited a concentration of 16,792.24 ± 3.78 µg/g, while freeze-dried aronia demonstrated a significantly higher content of 44,373.46 ± 3.21 µg/g, marking a notable 164.45% increase. This augmented total phenolic content is complemented by a 60% rise in anthocyanins, a 75% increase in flavonoids, and a 65% elevation in acids for freeze-dried aronia.

Table 3. The quantity of phenolic compounds in aronia samples (µg/g of sample).

Peak	Rt (min)	UV λmax (nm)	$[M + H]^+$ (*m*/*z*)	Compounds	Fresh Aronia [B]	Freeze-Dried Aronia [B]
1	3.04	270	139	Hydroxybenzoic acid	9198.83 ± 0.28	22,880.942 ± 1.20 ***
2	10.32	530, 280	611	Cyanidin diglucoside Delphinidin glucoside	3751.54 ± 0.35	9411.872 ± 0.27 ***
3	11.29	530, 280	465	Cyanidin glucoside	1203.94 ± 0.21	3010.610 ± 0.29 ***
4	13.05	529, 280	449	Cyanidin	124.38 ± 0.09	308.464 ± 0.10 ***
5	13.35	322	287	Caffeic acid	200.33 ± 0.11	764.026 ± 0.11 ***
6	14.48	356, 256	181	Kaempferol diglucoside	266.31 ± 0.10	978.302 ± 0.11 ***

Table 3. *Cont.*

Peak	Rt (min)	UV λmax (nm)	$[M + H]^+$ (*m*/*z*)	Compounds	Fresh Aronia [B]	Freeze-Dried Aronia [B]
7	14.88	360, 255	611	Quercetin-acetyl rhamnoside	331.80 ± 0.20	1068.519 ± 0.31 ***
8	15.61	360, 255	491	Quercetin-rutinoside (Rutin)	383.25 ± 0.33	1265.660 ± 0.41 ***
9	16.17	360, 255	611	Quercetin glucoside	581.29 ± 0.52	1700.039 ± 0.56 ***
10	18.75	356, 256	465	Kaempferol glucoside	272.54 ± 0.12	1269.002 ± 0.18 ***
11	21.37	360, 255	449	Quercetin	232.00 ± 0.18	974.960 ± 0.28 ***
12	23.27	356, 256	303	Kaempferol	246.03 ± 0.07	741.064 ± 0.29 ***
				Total Phenolics	16,792.24 ± 3.78	44,373.46 ± 3.21

The mean ± SD (n = 3) is provided in the descriptive statistics table. A two-way ANOVA was conducted to explore significant differences between fresh aronia and freeze-dried aronia by-product, followed by Tukey's multiple comparisons test. In this analysis, the second column was compared to the first column. The following symbols indicate different levels of significance: *** for $p < 0.001$, [B]—by-product.

In line with our findings, Lazăr et al. [30] underscored the exceptional value of aronia powder, attributing it to a rich composition of bioactive compounds, including high levels of total polyphenols, as well as vitamins C and E. Their study highlighted the antioxidant activity of the powder derived from *A. melanocarpa* pomace [30], emphasizing the intricate biochemical composition contributing to its noteworthy properties. The amplified presence of total phenolic compounds, along with anthocyanins, flavonoids, and acids in freeze-dried aronia, further positions it as a potent source of bioactive compounds with potential health benefits. This insight adds depth to our understanding of the nutritional composition of freeze-dried aronia, supporting its consideration as a valuable ingredient in functional foods and nutraceuticals.

3.3. In Vitro Digestion

To assess the release of nutrients, the cereal bar used as the model variant was enriched with freeze-dried *Aronia melanocarpa* by-product powder before and after the digestion process, and the plain freeze-dried powder was subjected to an in vitro digestion process. The quantity of phenolic compounds and anthocyanins in the samples is presented in Table 4.

Table 4. The quantity of phenolic and anthocyanin compounds in aronia by-product bar samples and an aronia bar before and after in vitro digestion (μg/g).

Peak	R_t (min)	Compound	Control Bar + Aronia before Digestion	Control Bar after Digestion	Control Bar + Aronia Powder after Digestion	Aronia Powder after Digestion
1	3.04	Hydroxybenzoic acid	223.58 ± 0.38	335.99 ± 0.78 [N.S.]	3592.40 ± 1.21 **	11,381.54 ± 4.27 *
2	3.63	Dihydroxybenzoic acid	n.d.	259.86 ± 0.52 [N.S.]	2828.27 ± 1.08 **	7125.05 ± 3.08 *
3	9.65	Protocatechuic acid	315.68 ± 0.41	482.72 ± 0.69 [N.S.]	3505.16 ± 1.57 **	11,548.09 ± 3.49 *
4	10.32	Cyanidin diglucoside Delphinidin glucoside	253.42 ± 0.30	n.d.	74.62 ± 0.18 **	5197.59 ± 1.36 *
5	11.29	Cyanidin-glucoside	101.53 ± 0.44	n.d.	71.99 ± 0.24 **	2696.02 ± 1.07 *
6	13.05	Cyanidin	29.49 ± 0.20	n.d.	n.d.	124.19 ± 0.19 *
7	13.35	Caffeic acid	40.69 ± 0.17	37.03 ± 0.20 [N.S.]	88.39 ± 0.32 **	1395.14 ± 1.01 *
8	14.48	Kaempferol diglucoside	30.96 ± 0.15	n.d.	40.49 ± 0.21 **	543.21 ± 0.78 *
9	14.88	Quercetin-acetyl-rhamnoside	37.31 ± 0.21	n.d.	38.01 ± 0.17 **	681.32 ± 0.86 *
10	15.61	Quercetin rutinoside (Rutin)	30.96 ± 0.18	n.d.	32.07 ± 0.14 **	624.89 ± 0.73 *
11	16.17	Quercetin glucoside	34.84 ± 0.14	n.d.	65.73 ± 0.29 **	540.24 ± 0.66 *
12	18.75	Kaempferol glucoside	42.25 ± 0.22	n.d.	n.d.	168.98 ± 0.31 *

Table 4. *Cont.*

Peak	R_t (min)	Compound	Control Bar + Aronia before Digestion	Control Bar after Digestion	Control Bar + Aronia Powder after Digestion	Aronia Powder after Digestion
13	21.37	Quercetin	32.37 ± 0.28	n.d.	n.d.	112.54 ± 0.09 *
14	23.27	Kaempferol	34.14 ± 0.11	n.d.	n.d.	102.15 ± 0.11 *
Total Phenolics			1207.22 ± 0.56	1115.61 ± 0.91	10,337.12 ± 1.49	42,240.94 ± 4.47
Total Anthocyanins			n.d.	384.44 ± 0.49	146.61 ± 0.28	8017.80 ± 1.91

The experiments underwent three replications, and the reported values represent the average and standard deviation (SD) of these replicates. Data normality was evaluated using the Shapiro–Wilk test, where a *p*-value greater than 0.05 indicated normally distributed data. The descriptive statistics table presents the mean ± SD (n = 3). A two-way ANOVA was performed to examine significant differences among the quantity of phenolic and anthocyanin compounds in aronia by-product bar samples before and after digestion, followed by Tukey's multiple comparisons test. In this analysis, each column was compared to the first column. Significance levels are indicated using the following symbols: ** for $p < 0.01$, * for $p < 0.05$, and $^{N.S.}$ (not significant), n.d.—not detected.

The aronia bar without powder has a very low amount of phenolic compounds (1115.61 ± 0.91 μg/g). Even more so, anthocyanins and flavonoids are not present. It contains 8% fewer phenolic compounds than the aronia bar enriched with powder before digestion, which had 1207.22 ± 0.56 μg/g. The aronia bar with powder has a modest amount of hydroxybenzoic acid, hihydroxybenzoic acid, and protocatechuic acid.

After digestion in the intestinal phase, phenolic compounds increased significantly in the aronia samples by 755.74% (10,337.12 ± 1.49 μg/g). This suggests that digestion can release phenolic compounds from aronia powder and contribute to better absorption in the body.

The aronia by-product had a phenolic compound content of (16,792.24 ± 3.78 μg/g), but their quantity was much higher after digestion in the intestinal phase of the lyophilized powder (44,373.46 ± 3.21 μg/g). Also, Lazăr et al. obtained a total of 8566–9693 mg GAE/g of polyphenols, concluding the benefits of the powder [30].

The difference between freeze-dried aronia before and after digestion is only −4.79%, indicating good absorption by the body even after digestion. This suggests that aronia is a significant source of phenolic compounds and that digestion can enhance the benefits of these compounds for health.

Anthocyanins were not detectable in the aronia bar before digestion. After digestion in the intestinal phase, anthocyanins were released in the aronia samples. Specifically, cyanidin diglucoside, delphinidin glucoside, cyanidin glucoside, and cyanidin were detected in significant quantities. This suggests that digestion can release anthocyanins from aronia powder, which can benefit their absorption in the body. In conclusion, adding aronia powder to cereal bars is an effective way to provide anthocyanins after digestion and increase the content of phenolic compounds, therefore offering antioxidant and health benefits.

Overall, these results suggest that consuming products enriched with aronia powder can contribute to the intake of beneficial nutritional and antioxidant compounds for health, while digestion can play a significant role in releasing these compounds in the body.

3.4. Nutritional Properties

The results of the nutritional analysis for the two bar variants, the control bar and the aronia bar, are presented in the table below.

The nutritional values of both bar variants exhibit a similarity, as can be observed in Table 5. This suggests that adding aronia to the bars' composition did not significantly impact their nutritional profile. Firstly, the control and aronia bars have a high dry matter content of 90% and a low water content of 10%, indicating good product stability and extended shelf life. These characteristics are essential for maintaining the quality of the product over the long term.

Table 5. The results of the nutritional analysis for the two bar variants.

	Control Bar	Bar with Aronia
Dry matter (%)	90.52 ± 1.22	90.43 ± 1.35 $^{N.S.}$
Humidity (%)	9.47 ± 0.55	9.56 ± 0.43 $^{N.S.}$
Ash (%)	1.82 ± 0.01	1.87 ± 0.10 $^{N.S.}$
Protein (%)	12.20 ± 0.28	12.28 ± 0.22 $^{N.S.}$
Fat (%)	20.51 ± 0.61	20.46 ± 0.35 $^{N.S.}$
Carbohydrates (%)	45.41 ± 0.26	45.47 ± 0.33 $^{N.S.}$
Fiber (%)	10.58 ± 0.47	10.35 ± 0.38 $^{N.S.}$
Kcal	429.87 ± 1.23	430.01 ± 1.56 $^{N.S.}$
Kj	1798.59 ± 2.96	1799.16 ± 3.09 $^{N.S.}$

The descriptive statistics table provides the mean ± SD (n = 3). A two-way ANOVA was conducted to explore significant differences between the control bar and bar with aronia, followed by Sidak's multiple comparisons tests. In this analysis, the second column was compared to the first column. The following symbols indicate different levels of significance: $^{N.S.}$ (not significant).

Regarding ash content, a minor difference was observed between the two variants, with the aronia bar having a slightly higher value of 0.05%. However, the discrepancy does not significantly influence the product's quality or organoleptic characteristics. The results were consistent with Blicharz-Kania et al. [31], which emphasises that including dried fruit (20%) in bars does not significantly impact nutritional characteristics. Also, Munhoz et al. [32] found that a cereal bar containing bocaiuva had an ash ratio of 1.29%, indicating that ash concentration may range from 0.3% to 30%, depending on the mineral composition of the cereals.

The protein and fat content were very close in both bars, at 12% and 20%, respectively, suggesting that adding aronia had no significant impact on the macronutrient content. The protein level can have a significant impact on the texture, structure, and shelf stability of the finished product [33,34]. For example, a protein content higher than 20% leads to a denser and more substantial texture in the bars, affecting their acceptability by consumers [33]. On the other hand, the high fat content suggests a greater caloric density, providing a more concentrated energy source. This may be attributed to the significant quantity of cashew butter used in the bars. Cashew butter contains predominantly unsaturated fats, including monounsaturated and polyunsaturated fatty acids, which have been reported to have antioxidant properties [35]. These antioxidants help protect the fats in cashew butter from oxidation, thereby minimizing the development of off-flavours and rancidity [34]. Thus, adding cashew butter to the cereal bars improves their taste stability by keeping the flavour fresh and preventing the risk of rancidity and off-flavours.

Additionally, the carbohydrate and fibre content was similar in both variants, at 45% and 10%, respectively, while the calorie intake was almost identical at 430 kcal. This indicates that the aronia bar offers the same caloric and nutritional benefits as the control bar without making significant changes.

Alfheeaid et al. [36] conclude the nutritional and functional benefits of snack bars. They created two cereal bars with added dried fruits and dates. Both had a similar nutritional profile and caloric intake: protein 21%, moisture 11–13%, total fat 6–7%, fibre 8–9%, carbohydrates 55–57%, 376–378 kcal per 100 g [36]. Similarly, another study conducted by Szydłowska et al. [37] suggests that adding freeze-dried raspberries (0.8%) extended the shelf life of protein snack bars but contributed no significant nutritional quality.

3.5. Influence of Time on Microbiological Analysis

Figure 3 presents the microbiological analysis of bars supplemented with aronia by-products and control bars (without aronia by-products). These bars are crafted with ingredients such as cashew butter, honey, oat flakes, and coconut flakes, and they are supplemented with aronia by-product. Throughout a three-month storage period at room temperature, samples were taken weekly for microbiological analysis. In addition to assessing sensory quality, textural characteristics, and nutritional value, evaluating microbi-

ological quality is crucial for ensuring consumer health and safety. Therefore, the bars were assessed against microbiological quality parameters before conducting sensory tests [38].

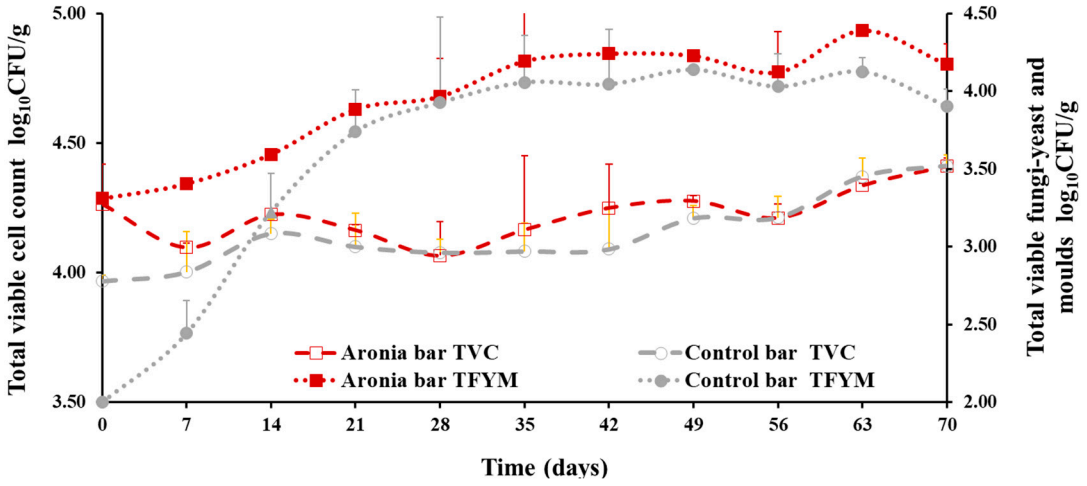

Figure 3. Microbiological analysis of control bar and bar with aronia pomace during 70 days; TVC—total viable cell count, TFYM—total viable fungi-yeast and moulds.

The TVC increased from 4.26 ± 0.02 $\log_{10}$ CFU/g to 4.41 ± 0.03 $\log_{10}$ CFU/g for aronia bars and from 3.97 ± 0.02 $\log_{10}$ CFU/g to 4.41 ± 0.04 $\log_{10}$ CFU/g for control bars. Similarly, TFYM increased from 3.31 ± 0.22 $\log_{10}$ CFU/g to 4.17 ± 0.13 $\log_{10}$ CFU/g for aronia bars and from 2.00 ± 0.01 $\log_{10}$ CFU/g to 3.91 ± 0.11 $\log_{10}$ CFU/g for control bars. Throughout storage, there was an increase in TVC and TFYM in all samples, but the changes were not substantial. To control the growth of TFYM, the application of vacuum or modified atmosphere packaging is recommended.

In comparison with the study by Szydłowska et al. [37], where a bar supplemented with whey protein and bioactive ingredients showed TVC results between 3.00 and 4.85 $\log_{10}$ CFU/g and TFYM less than 3 $\log_{10}$ CFU/g, our results are within a similar range. Conversely, Munshi et al. [39] reported the highest TVC (8.4×10^3 CFU/g) in their study [39]. Additionally, after screening bars for their aerobic mesophilic count (CFU/g) and yeast and mould count (CFU/g) during a 90-day storage period, Munshi et al. [39] found a high aerobic mesophilic count (8.4×10^3 CFU/g) and 3.6×10^1 CFU/g of yeasts and moulds in multi-nutrient bars [39]. Also, Veggi et al. [40] showed excellent microbiological quality (total coliforms, yeasts, and mould counts $<5.0 \times 10^1$ CFU/g) in high-protein diet bars with the addition of chia grain [40].

In our present manuscript, the decision to formulate only two types of bars was influenced by a previous study by Has et al. [28] conducted by the group. The prior research focused on developing oat- and millet-based snack bars enriched with freeze-dried elderberry powder, aiming to enhance their nutritional value, antioxidant properties, and prebiotic potential. The study encompassed sensory evaluation, nutritional assessment, and rheological analysis of the snack bars. Given the comprehensive assessment conducted in the previous study, the current investigation focused on in vitro digestion and nutritional quality evaluation of the bars enriched with *A. melanocarpa* pomace powder, hence the decision to analyse only two variants.

4. Conclusions

In conclusion, the integration of *A. melanocarpa* pomace powder into bars has unveiled a realm of possibilities for sustainable and nutritious food development. The significant increase in antioxidant activity (61%), coupled with the maintenance of essential nutritional values, underscores the potential of these bars as a valuable source of bioactive compounds.

The findings reveal that aronia-enriched bars maintain consistent nutritional values, with protein and fat content remaining at approximately 12% and 20%, respectively. More-

over, this innovation contributes to a calorie reduction, resulting in bars with 430 kcal, which is of benefit to consumers. Additionally, there is a notable 755.74% increase in phenolic compounds after the digestion phase.

Furthermore, both types of bars exhibited a comparable value of 4.41 $\log_{10}$ CFU/g for viable cells after 70 days of room temperature storage. However, the presence of fungi, yeast, and moulds increased to 4.17 $\pm$ 0.13 $\log_{10}$ CFU/g in aronia bars and 3.91 $\pm$ 0.11 $\log_{10}$ CFU/g in control bars after the same storage period.

By repurposing by-products and reducing calorie content without compromising on quality, this innovative approach not only addresses consumer health needs but also promotes eco-friendly practices in the food industry. The findings of this study advocate for the widespread adoption of such practices to harness the nutritional and ecological benefits of utilizing *A. melanocarpa* by-products in functional food products. This work presents limitations, such as the absence of any specific analysis of dietary fibre, phenolic compound, and antioxidant activity over storage tests. Additionally, future experiments could benefit from further analyses of a range of additive concentrations to optimise the nutritional and physical-chemical interactions, such as colour, water activity, pH, acidity, and functional properties of the bars.

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