

Article

Influence of Sea Buckthorn Fruit Part on Physical, Quality and Bioactive Properties of White Chocolate Under the Circular Economic Framework

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Abstract

The addition of sea buckthorn (*Hippophae rhamnoides* L.) fruits as well as their extracted juice or, even more interestingly, related by-products into chocolate results in manufacturing an innovative functional food rich in bioactive substances. Thirteen treatments derived from the factorial combination of three types of *H. rhamnoides* materials (total fruit powder; fruit by-product powder; and fruit juice) and four concentrations (10%, 15%, 20% and 25%), plus an untreated control, were compared in terms of texture, quality, colour, antioxidant, mineral and sensorial properties of white chocolate. The untreated control showed the highest values of most of the texture parameters, as well as of pH, dry matter, soluble solids and colour component ‘L’. The colour component ‘b’ was best influenced by the 10% by-product addition to chocolate, whereas mineral substances, ash and colour component ‘a’ augmented with the increasing concentration of added *H. rhamnoides* materials. Compared to the untreated control, protein and fat contents in chocolate decreased with the rising added concentration of sea buckthorn fruit juice but showed the opposite trend under the integration of the whole fruit and its by-products. The antioxidant compounds and activity increased from the untreated chocolate to the highest concentration of added sea buckthorn materials. The juice addition to the chocolate best affected vitamin C, total carotenoids, β -carotene and lycopene, whereas the whole fruit integration led to the top levels of flavonoids, polyphenols and antioxidant activity. Potassium and zinc contents decreased from the untreated control to the highest *H. rhamnoides* material addition, whereas opposite trends were shown by calcium, magnesium, sodium and phosphorus. The integration of *H. rhamnoides* fruit materials into chocolate presents a valuable strategy to produce innovative health beneficial functional food.

Keywords: *Hippophae rhamnoides* L.; functional food; innovative products; texture; colour; antioxidants; mineral composition



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1. Introduction

Consumer demand for functional foods [1,2] has been increasing over the last decades due to the health-beneficial effects of these products rich in bioactive compounds [3]. In-

deed, manufacturing innovative foods has modernized the confectionery industry, providing new attractive proposals of quality and sensory characteristics to meet the requirements of consumers [4].

The use of sea buckthorn fruits to produce original products led to successful solutions, such as novel milk-derived items [3], jam, jelly and drinks [5]. Additionally, several antioxidants, either phenolics or plant extracts, have been inserted inside chocolate chains, and the replacement of sugar with various plant mixtures has contributed to preventing many diseases [6–13]. Plant-derived polyphenols added to chocolate, from carob [6] or *Hippophae rhamnoides* materials, showed beneficial antimicrobial and antioxidant activity to manufacture premium-quality natural foods. Particularly, white chocolate is derived from the combination of cocoa butter, milk or milk products and sugars, containing the following: $\geq 20\%$ cocoa butter, $\geq 14\%$ dry milk solids and at least 3.5% milk fat [14].

Hippophae rhamnoides is a shrub of Elaeagnaceae family, widely distributed in Asia and Europe [15], growing well even in poor and dry soils [16], and its fruits are very health beneficial [17,18].

Hippophae rhamnoides fruits have a high content of antioxidants and therapeutic substances, like the flavonoids rutin, quercetin, kaempferol, myricetin and isorhamnetin [19], tocopherols and carotenoids [20], L-ascorbic acid, volatile oils, vitamins, amino acids and minerals [21], and have remarkable antioxidant activity [22]. Sea buckthorn fruits are rich in essential healthy compounds, showing nutraceutical effects against inflammation and toxicity, and foster hair and skin regrowth [23]. The remarkable concentrations of vitamin C and n-3, n-6 and n-9 polyunsaturated fatty acids make it beneficial to integrate *Hippophae rhamnoides* in several foods, like juice, alcohol beverages like wine, liquer and beer additive, as well as jam, jelly, marmalade, sauce, oil, syrup, soft drinks [24], freeze-dried powder, milk tablets, tea and preserved fruit [5].

As the mentioned manufacturing chains give rise to high amounts of waste and by-products [23], the present study has been planned to valorise the sea buckthorn industry by-products from the perspective of sustainably managing the food systems and to solve the possible lack of related resources. In this respect, our target of preventing waste's negative environmental impacts coincides with that of the European Green Deal [25], and, additionally, the sea buckthorn industry can be boosted [18].

In this research, sea buckthorn powder and juice by-products have been used as they are both versatile products with therapeutic and antioxidant properties that can be effectively employed for manufacturing innovative chocolate products with improved quality and nutritional characteristics. The formulation of the mentioned waste derived from the sea buckthorn fruit pressing to obtain juice at the Elexius unit, in combination with the concentration and the presence or absence of oil, was assessed to identify the best performing recipe of a sea buckthorn-added novel chocolate, in terms of texture, quality, colour, antioxidant and mineral characteristics.

2. Materials and Methods

2.1. Experimental Protocol and Raw Materials

Research was conducted at Iasi University 'Ion Ionescu de la Brad' in 2025 to compare thirteen treatments derived by the factorial combination of 3 types of sea buckthorn (*Hippophae rhamnoides* L.) material (total fruit powder; fruit by-product powder; and fruit juice), and 4 concentrations of addition to white chocolate (10%; 15%; 20%; and 25%), plus an untreated control, using a randomized complete block design with three replicates, in terms of the following: texture, quality, colourimetric, antioxidant, mineral and sensorial characteristics of chocolate.

Sea buckthorn berries (SBB—*Hippophae rhamnoides* L.), grown in organic conditions, were used entirely or as juice extracted by Elexius, manufactured in the Bacău region (Romania), or as by-products following the mentioned extraction, to produce innovative white chocolate. The premium quality of *Hippophae rhamnoides* L. fruits relates to the high levels of antioxidant compounds and capacity, lipids and proteins, mineral elements, physical-chemical and colourimetric characteristics [23].

2.2. Sea Buckthorn Material, Fruit Processing, Chocolate Preparation and Processing

The fruits were harvested in August, quickly frozen (in 27 min) at -40°C , then stored until processing (about 3 months) at -25°C .

The fruit processing was carried out as previously described [26,27].

White chocolate is melted in the mouth and, to avoid perceiving solid particles, the latter must be smaller than $20\text{--}25\text{ }\mu\text{m}$ (the threshold for olfactory organ detection); accordingly, pleasant aroma and taste arise upon processing the main ingredients such as milk and sea buckthorn powder, cocoa butter, sugar and flavourings.

In the present research, the 12 experimental chocolate samples, plus the untreated control, were manufactured in the form of bars, referring to the international classification [26]. Chocolate quality is correlated with the particle fineness of *H. rhamnoides* powder, sugar and possibly milk powder; moreover, its unctuousness, taste and smell also depend on fat content and flavouring.

The chocolate processing has been managed as previously described [26].

The chocolate samples were obtained according to the recipes described in Table 1, including the following raw materials: Rarăul[®] milk powder; Bio&Raw[®] cocoa butter; Delmario[®] sugar; water; and sea buckthorn fruit parts, i.e., concentrated juice, whole fruit or by-product powder derived from the juice extraction carried out at the Elexius[®] factory (Table 2).

Table 1. Recipes used to obtain the untreated control and *H. rhamnoides*-enriched chocolate samples.

Experimental Treatment	Milk Powder %	Caster Sugar %	Water %	Cocoa Butter %	Sea Buckthorn %
Chocolate with no additions	45	20	15	20	-
Chocolate with 10% juice addition	35	20	15	20	10
Chocolate with 15% juice addition	30	20	15	20	15
Chocolate with 20% juice addition	25	20	15	20	20
Chocolate with 25% juice addition	20	20	15	20	25
Chocolate with 10% fruit addition	35	20	15	20	10
Chocolate with 15% fruit addition	30	20	15	20	15
Chocolate with 20% fruit addition	25	20	15	20	20
Chocolate with 25% fruit addition	20	20	15	20	25
Chocolate with 10% by-product addition	35	20	15	20	10
Chocolate with 15% by-product addition	30	20	15	20	15
Chocolate with 20% by-product addition	25	20	15	20	20
Chocolate with 25% by-product addition	20	20	15	20	25

Table 2. Chemical composition of *H. rhamnoides* fruit parts.

<i>H. rhamnoides</i> Fruit Part	DM %	Vit C mg 100 g ^{−1}	Fl mg CE g ^{−1}	Pol mg GAE g ^{−1}	AA μmol Trolox eq. g ^{−1}	TC mg g ^{−1}	β-car mg g ^{−1}	Lyc mg g ^{−1}	Pr mg g ^{−1}	Lip mg g ^{−1}	K	Ca	Mg	Na	P	Zn
mg 100 g ^{−1}																
By-product	96.7	1300.6	2.3	5.0	22.6	1.0	0.6	0.2	150.4	240.6	56.3	43.6	64.7	33.8	61.0	1.36
Whole fruit	14.0	550.8	3.4	16.3	119.6	42.1	28.4	4.9	120.3	196.2	67.6	37.5	56.0	40.3	53.5	1.45
Juice	10.0	127.6	4.1	22.1	174.8	62.7	43.3	7.8	102.7	169.5	70.5	35.2	53.4	42.1	50.9	1.48

DM: Dry matter; Vit C: Vitamin C; Fl: Flavonoids; Pol: Polyphenol; AA: Antioxidant activity; TC: Total carotenoids; β-car: β-carotene; Lyc: Lycopene; Pr: Protein.; Lip: Lipid. The quality and antioxidant compounds and the antioxidant activity are expressed per fresh weight, and the mineral elements per dry weight.

2.3. Determination of Textural Properties

The texture of the chocolate samples added with sea buckthorn fruit was determined by the Mark 10[®] texturometer (Copiague, NY, USA). In this respect, the cylindrical probe type TA5, with a diameter of 12.7 mm and a height of 35 mm, was used for compression tests, and the Warner Bratzler knife-type probe for cutting tests of the chocolate samples. The probes were attached to an M5-50E dynamometer with a maximum load capacity of 250 N and a resolution of 0.05 N. The movement speed of the probes in the chocolate samples was 2 mm s^{−1} for all tests. The force–strain and force–time graphs obtained experimentally were recorded in a computer with the MeasurePlus software model 15-1006 (Copiague, NY, USA). The results obtained from the graphs related to the mentioned texture parameters were calculated using GraphPad Prism 9 and Microsoft Office Excel 2021 software. To determine the texture characteristics of each chocolate sample in triplicate, three bars with the following geometric characteristics were used: 22 ± 1 mm height, 87 ± 1 mm length and 37 ± 1 mm width. The texture of all sea buckthorn chocolate samples was tested at a temperature of 20 ± 1 °C, and, therefore, the samples remained solid throughout the experiment. In the compression tests, the TA5 probe penetrated the chocolate sample through 10 mm at a constant speed of 2 mm s^{−1}.

The texture tests of the sea buckthorn chocolate samples were performed to determine the elastic–plastic behaviour and the maximum force required for their compression. The physical parameters were determined using the mentioned cylindrical probe and the force–displacement graph. The coefficient *K* related to the elastic–plastic behaviour of the chocolate samples subjected to compression stress was calculated as follows:

$$k = \frac{Le}{Lp} \quad (1)$$

where *Le* (mJ) is the elastic mechanical work; *Lp* (mJ) is the plastic mechanical work. The coefficient *k* values range between 0 and 1, where 0 represents the state of pure plasticity and 1 the state of pure elasticity.

The cutting force of the chocolate samples added with sea buckthorn fruit materials was determined by the Warner Bratzler knife-type probe and derived from the force–displacement graph.

The mechanical texture characteristics were obtained from the force–time graphs, where a double compression stress is performed with the cylindrical probe, according to TPA (Texture Profile Analysis) [28,29].

The mentioned texture determinations were repeated three months after the production of *Hippophae rhamnoides*-added chocolate to assess the stability of the product, but the results have not been reported because they are not significantly different from those recorded at the first determination.

2.4. Determination of Total Dry Matter Content

The total dry matter content was determined as previously reported [30].

The dry matter determination, as well as all the following quality analyses, were repeated three months after the production of *Hippophae rhamnoides*-added chocolate, but the results have not been reported because they are not significantly different from those recorded at the first determination.

2.5. Determination of Protein and Crude Fat Contents

Nitrogen, protein and crude fat contents were measured according to published methods [30–32].

2.6. Determination of the Colour Components

The colour components L^* , a^* and b^* were determined on the chocolate sample surface, as reported by other authors [33].

2.7. Extraction of Bioactive Substances from Chocolate Samples, and Determination of Vitamin C, Flavonoid, Total Polyphenol and Carotenoid Contents

The phytochemicals from the white chocolate samples with the addition of sea buckthorn powder were extracted as previously described [26]. Vitamin C was extracted and analyzed according to published methods [26,34]. The determination of total flavonoids (TFC) was performed using the spectrophotometric method based on the reaction between aluminum chloride and phenolic compounds, as previously described [26]. The content of carotenoids (β -carotene and lycopene) was determined according to published methods [33], and the total polyphenols were measured using Folin–Ciocâlteu's reagent method [35].

The following antioxidant determinations were repeated three months after the production of *Hippophae ramnoides*-added chocolate, but the results have not been reported because they are not significantly different from those recorded at the first determination.

2.8. Determination of Antioxidant Activity

The antioxidant activity was determined as previously published [36].

2.9. Determination of Mineral Elements

The mineral element contents (K, Ca, Mg, Na, P, Zn, and Fe) of the white chocolate samples added with sea buckthorn were measured according to other authors' description [26].

2.10. Sensory Features

The sensory features were evaluated based on the previously reported description [26,37].

2.11. Statistical Analysis

Data were obtained in duplicate, processed by analysis of variance (ANOVA), with three replicates, and mean separations were performed through Duncan's test, with reference to a 0.05 probability level, using SPSS software version 29. The results are presented as mean $\pm$ standard deviation.

3. Results and Discussion

3.1. Texture Parameters

The texture characteristics of the chocolate (Tables 3 and 4) show a maximum compression force in the samples without *R. ramnoides* fruit part addition, and, upon raising the concentration of both the added juice and fruit, it decreased as a result of the rigidity decrease in the chocolate samples. The maximum compression force was higher in the chocolate samples added with 10% fruit compared to those added with 10% juice, but lower than the untreated control. The latter differences can be explained by the decrease in the product rigidity upon increasing the concentration of added juice or fruit into the chocolate samples. The highest values of cutting force were recorded in the untreated control and decreased with the juice or fruit addition, ranging from a minimum of 7.7 N to a maximum of 23.3 N; therefore, the samples fall into the category of soft products with variation between 0.1 N and 30 N.

The k coefficient, showing how the chocolate texture turns from the elastic to the plastic range, was 0.63 for the untreated chocolate (into the elastic–plastic range) and

displayed a plastic behaviour upon the sea buckthorn juice or fruit addition, decreasing down to a minimum value of 0.03.

The texture characteristics resulting from Texture Profile Analysis (TPA) obtained from the force–time graph are presented in Table 2. Hardness is a mechanical characteristic, defined as the resistance of chocolate to compressive force, and from the force–time graph, it represents the maximum force resulting in the first cycle of compressive load. The hardness value is maximum in the untreated chocolate and gradually decreases with the rise in the percentages of sea buckthorn fruit, powder and juice, and the latter showing the lowest level, presumably due to the increase in moisture content.

Consistency represents the total energy absorbed during the mastication or handling process (compression load) and is generally evaluated as the area under the first compression curve. A product with low consistency, such as soft chocolate, deforms more easily compared to those with a higher consistency and more compact structure, e.g., a firm jelly, because it requires lower energy for deformation. The consistency or energy required for deformation decreased from the untreated chocolate to the highest *H. rhamnoides* fruit material addition as a result of the dry matter content decrease.

Adhesion is the characteristic showing the energy needed to overcome the forces of attraction between the chocolate and the probe surface it comes into contact with. In this respect, chocolate can be sticky, with stronger or weaker adhesion, and in the present research, adhesion is the negative area in the force–time graph obtained after the first compression of the chocolate sample; the higher the value of this area, the stronger the adhesion. The latter values increased from the untreated control (1.03 mJ) to the highest sea buckthorn fruit by-product addition (2.91 mJ), which demonstrated that the integrated powder had a strong adhesive impact on the chocolate's basic structure.

Cohesion is the texture characteristic showing the strength reaction of chocolate samples to the second deformation in relation to the first deformation strength by compressive stress. The mentioned behaviour expresses the strength degree of the internal bonds of the product structure, with cohesiveness values ranging between 0 and 1, where 0 is associated with very weak internal bonds and 1 with very strong ones. In this respect, the untreated control attained a value of 0.147, and with the rising addition of sea buckthorn fruit parts, the cohesiveness increased, reaching the highest value of 0.427, associated with the by-product powder due to its binder role in the chocolate structure.

Elasticity, representing the degree of the chocolate physically recovered after the second compression deformation cycle compared to the first one, showed the highest values in the untreated control or under low concentrations of *H. rhamnoides* fruit material integration; the mentioned parameter is positively correlated with consistency.

Chewability, an important parameter of solid chocolate, is the measure of chewing force required to break the product into smaller pieces, and reached the top value of 8.10 N in the untreated chocolate and decreased upon the addition of sea buckthorn fruit parts, with the lowest value of 2.53 N caused by the integration of 25% juice.

In previous research [9], the addition of microencapsulated or microalgae containing forms of omega 3 fatty acids (EPA/DHA) did not have a strong impact on texture characteristics of chocolate, particularly plastic viscosity, and did not show a manufacturing process change. The highest chocolate yield stress and plastic viscosity corresponded to the microencapsulated form. Notably, the omega 3 integration led to the shear-thinning behaviour of chocolate, likely due to the structural breakdown occurring upon the applied shear force [38] and alignment of the constituent molecules [39].

Table 3. Texture characteristics of chocolate added with *H. rhamnoides* fruit materials.

Type of <i>Hippophae rhamnoides</i> Fruit Material (TP) × Percentage of Addition (PA)	Fmax Compression (N)	k Elasto–Plastic Coefficient (-)	Fmax Cutting (N)
Chocolate with no additions	37.6 ± 2.0 a	0.63 ± 0.03 a	23.3 ± 1.9 a
Chocolate with 10% juice addition	20.1 ± 1.7 e	0.18 ± 0.04 cd	20.4 ± 1.2 bc
Chocolate with 15% juice addition	16.6 ± 1.2 f	0.13 ± 0.10 e	11.7 ± 0.8 d
Chocolate with 20% juice addition	12.7 ± 1.5 g	0.05 ± 0.01 f	10.7 ± 1.3 df
Chocolate with 25% juice addition	12.7 ± 0.5 g	0.05 ± 0.01 f	9.4 ± 0.8 eg
Chocolate with 10% fruit addition	30.2 ± 1.0 b	0.20 ± 0.01 c	21.9 ± 1.7 ab
Chocolate with 15% fruit addition	14.8 ± 1.0 f	0.15 ± 0.01 de	18.6 ± 1.2 c
Chocolate with 20% fruit addition	10.3 ± 0.8 h	0.04 ± 0.01 f	10.9 ± 1.2 de
Chocolate with 25% fruit addition	9.2 ± 0.1 h	0.03 ± 0.01 f	10.7 ± 1.0 df
Chocolate with 10% by-product addition	37.2 ± 0.5 a	0.26 ± 0.02 b	22.6 ± 1.3 a
Chocolate with 15% by-product addition	26.3 ± 1.5 c	0.15 ± 0.01 de	12.0 ± 0.6 d
Chocolate with 20% by-product addition	15.3 ± 0.3 f	0.04 ± 0.01 f	8.8 ± 0.7 fg
Chocolate with 25% by-product addition	10.8 ± 0.9 h	0.04 ± 0.01 f	7.7 ± 0.9 g

n.s.: not significant; within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

Table 4. Texture characteristics (TPA) of chocolate added with *H. rhamnoides* fruit materials.

Type of <i>Hippophae rhamnoides</i> Fruit Material (TP) × Percentage of Addition (PA)	Hardness (N)	Consistency (mJ)	Adhesiveness (mJ)	Cohesiveness (-)	Elasticity (-)	Chewability (N)
Chocolate with no additions	28.1 ± 0.7 a	184.5 ± 8.4 a	1.03 ± 0.12 i	0.147 ± 0.015 g	1.74 ± 0.10 a	8.10 ± 0.63 a
Chocolate with 10% juice addition	14.1 ± 2.0 de	95.6 ± 10.0 e	1.19 ± 0.10 hi	0.161 ± 0.010 g	1.31 ± 0.10 b	4.61 ± 0.29 f
Chocolate with 15% juice addition	12.4 ± 1.5 ef	80.7 ± 8.0 f	1.33 ± 0.05 h	0.214 ± 0.010 f	1.07 ± 0.10 df	4.28 ± 0.29 fg
Chocolate with 20% juice addition	9.9 ± 0.6 gh	65.9 ± 4.0 g	1.50 ± 0.16 g	0.221 ± 0.010 f	1.06 ± 0.05 ef	3.02 ± 0.20 h
Chocolate with 25% juice addition	9.7 ± 0.6 h	57.0 ± 3.0 g	1.72 ± 0.10 f	0.297 ± 0.020 de	0.95 ± 0.05 f	2.53 ± 0.10 i
Chocolate with 10% fruit addition	18.1 ± 0.7 c	142.3 ± 5.0 bc	1.29 ± 0.05 h	0.214 ± 0.010 f	1.69 ± 0.07 a	7.19 ± 0.20 c
Chocolate with 15% fruit addition	14.6 ± 0.6 d	139.0 ± 4.0 c	1.44 ± 0.02 gh	0.285 ± 0.015 e	1.34 ± 0.05 b	5.43 ± 0.20 e
Chocolate with 20% fruit addition	13.5 ± 0.5 de	130.7 ± 3.0 c	2.01 ± 0.10 e	0.310 ± 0.020 cd	1.23 ± 0.05 bc	3.32 ± 0.10 h
Chocolate with 25% fruit addition	10.2 ± 0.5 gh	107.8 ± 3.0 d	2.38 ± 0.10 bc	0.338 ± 0.020 b	1.15 ± 0.05 ce	2.69 ± 0.10 i
Chocolate with 10% by-product addition	26.9 ± 1.0 a	180.2 ± 7.0 a	2.19 ± 0.10 d	0.288 ± 0.020 de	1.74 ± 0.10 a	7.63 ± 0.30 b
Chocolate with 15% by-product addition	21.2 ± 0.8 b	153.0 ± 6.0 b	2.23 ± 0.1 cd	0.292 ± 0.015 de	1.66 ± 0.10 a	6.52 ± 0.20 d
Chocolate with 20% by-product addition	12.6 ± 0.7 ef	92.9 ± 7.0 e	2.51 ± 0.13 b	0.321 ± 0.017 bc	1.19 ± 0.05 cd	4.06 ± 0.20 g
Chocolate with 25% by-product addition	11.5 ± 0.5 fg	64.1 ± 6.0 g	2.91 ± 0.15 a	0.427 ± 0.020 a	0.95 ± 0.05 f	2.57 ± 0.10 i

n.s.: not significant; within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

Chocolate texture properties [40] and plastic viscosity [41] influence the efficiency of the production chain, and, particularly, yield stress is affected by material surface and structure [42]. The manufacturing system modulates chocolate hardness as well as the ingredients, among which fats and sugars determine softness [43]. The relation between hardness and fatty acid profile, reported in form V of cocoa butter [43], relates to the crystallization of fat triglycerides in triple chains with greater thermodynamic stability compared to the double ones [42].

In a previous investigation [9], the addition of omega 3 fatty acids to chocolate reduced its hardness, with negligible effect in terms of melting properties and temperature, the latter showing inverse correlation with the polyunsaturated fatty acid content (PUFA).

In a previous study [10], sugars were replaced with inulin and maltitol to produce an innovative health-beneficial chocolate as a no-palm-oil functional food fortified with nanoparticles of vitamin D and magnesium–calcium carbonate; the latter enhanced the viscosity, similarly to the water content increase presumably fostered by nanoparticles. The lower value of viscosity, compared to that recorded in palm oil and sugar-added chocolate, was likely due to the 33% fat content. The consistency index showed the same trend as plastic viscosity and yield stress, the latter influenced by interparticle interactions [44], which are reduced by the hydrophobic vitamin D addition into the formulation because of the higher fat-like material content coating all the particles [10]. The integration of Vitamin D + calcium into no-palm-no-sugar chocolate led to lower viscosity compared to the Ca addition [10] due to enhanced interparticle interactions consequent to lower availability of fats coating them [45].

3.2. Quality and Colour Parameters

Regarding the quality characteristics of chocolate added with *Hippophae rhamnoides* materials (Table 5), the untreated control showed the highest values of pH, dry matter, soluble solids and colour component 'L'; the mentioned parameters displayed decreasing trends with rising concentration of the integrated sea buckthorn parts. A similar trend was recorded for the colour component 'b', but the highest value was obtained upon the treatment with the 10% fruit by-product. On the contrary, mineral substances, ash and colour component 'a' augmented with increasing concentration of added *H. rhamnoides* material. Compared to the untreated control, protein and fat contents in chocolate showed decreasing values with the rise in added concentration of sea buckthorn fruit juice, whereas the integration of the whole fruit and its by-products led to the opposite trend.

The decreasing pH, dry matter and soluble solids trend of chocolate upon the addition of *H. rhamnoides* materials is due to the lower values of this parameter recorded in all the sea buckthorn fruit parts integrated (Table 5); the increasing trends of protein and fat content under the inclusion of sea buckthorn whole fruit or its by-product relates to the higher content of these substances compared to chocolate, contrary to what is recorded about the fruit juice addition. In previous research [46], the addition of powder derived from black currant or blueberry fruits, *Hibiscus* flowers and cloves buds to chocolate resulted in similar protein content as in the present research, but lower fats and higher mineral substances.

Table 5. Quality and colour characteristics of chocolate added with *H. rhamnoides* fruit materials.

Type of <i>Hippophae rhamnoides</i> Fruit Material (TP) × Percentage of Addition (PA)	Dry Matter (%)	Soluble Solids (°Brix)	pH	Proteins	Fats	Mineral Substances	Ash	L*	a*	b*
Chocolate with no additions	91.0 ± 1.2 a	7.0 ± 0.2 a	6.67 ± 0.02 a	11.1 ± cf	18.5 ± fg	2.2 ± 0.1 d	0.08 ± 0.01 bc	88.3 ± 2.1 a	2.30 ± 0.5 g	35.5 ± 1.4 b
Chocolate with 10% juice addition	89.2 ± 1.5 ac	5.0 ± 0.1 b	5.79 ± 0.02 c	10.9 ± dg	18.2 ± g	2.5 ± 0.1 bc	0.08 ± 0.01 bc	86.1 ± 0.6 ab	3.51 ± 0.2 g	25.4 ± 0.5 cd
Chocolate with 15% juice addition	88.2 ± 2.0 ad	4.9 ± 0.1 b	5.54 ± 0.01 e	10.8 ± eg	18.0 ± g	2.6 ± 0.2 ab	0.08 ± 0.01 bc	85.2 ± 0.4 ab	4.02 ± 0.1 f	24.5 ± 0.4 d
Chocolate with 20% juice addition	86.9 ± 1.5 bd	4.8 ± 0.2 bc	5.01 ± 0.02 f	10.7 ± fg	17.7 ± g	2.6 ± 0.3 ab	0.09 ± 0.01 ac	84.9 ± 0.1 b	4.56 ± 0.1 e	22.4 ± 0.4 e
Chocolate with 25% juice addition	85.9 ± 1.8 cd	4.8 ± 0.1 bc	4.80 ± 0.04 g	10.5 ± g	17.5 ± g	2.6 ± 0.4 ab	0.09 ± 0.02 b	83.7 ± 1.3 b	4.63 ± 0.1 e	20.5 ± 0.2 ef
Chocolate with 10% fruit addition	89.7 ± 2.0 ab	5.0 ± 0.2 b	5.79 ± 0.06 c	11.3 ± ce	19.6 ± ef	2.4 ± 0.4 cd	0.07 ± 0.02 c	70.3 ± 0.3 c	4.76 ± 0.1 e	27.2 ± 0.3 c
Chocolate with 15% fruit addition	88.7 ± 1.3 ac	4.9 ± 0.2 b	5.70 ± 0.01 d	11.4 ± bd	20.5 ± ce	2.4 ± 0.2 cd	0.08 ± 0.01 bc	64.8 ± 0.1 d	5.46 ± 0.1 d	26.3 ± 0.3 cd
Chocolate with 20% fruit addition	84.8 ± 2.0 d	4.6 ± 0.1 c	5.56 ± 0.02 e	11.6 ± bc	21.3 ± cd	2.7 ± 0.3 ab	0.08 ± 0.02 bc	57.7 ± 0.6 e	6.35 ± 0.1 c	22.2 ± 0.7 e
Chocolate with 25% fruit addition	84.7 ± 1.7 d	4.4 ± 0.1 d	5.56 ± 0.02 e	11.9 ± ab	22.8 ± b	2.9 ± 0.4 ab	0.09 ± 0.01 ab	55.4 ± 0.1 e	6.90 ± 0.1 b	19.7 ± 0.3 f
Chocolate with 10% by-product addition	90.2 ± 1.6 ab	5.0 ± 0.2 b	5.86 ± 0.02 b	11.4 ± bd	20.3 ± de	2.3 ± 0.2 cd	0.08 ± 0.01 bc	69.2 ± 0.9 c	5.43 ± 0.3 d	39.4 ± 1.2 a
Chocolate with 15% by-product addition	89.7 ± 2.0 ab	4.9 ± 0.1 b	5.53 ± 0.02 e	11.6 ± bc	21.5 ± c	2.4 ± 0.1 cd	0.08 ± 0.01 bc	66.2 ± 1.2 d	6.42 ± 0.1 c	36.5 ± 0.7 b
Chocolate with 20% by-product addition	89.5 ± 2.0 ac	4.9 ± 0.2 b	5.02 ± 0.02 f	11.9 ± ab	23.0 ± b	2.5 ± 0.1 bc	0.08 ± 0.01 bc	57.4 ± 0.2 e	7.81 ± 0.5 a	35.7 ± 0.8 b
Chocolate with 25% by-product addition	87.2 ± 1.8 ad	4.8 ± 0.1 bc	4.81 ± 0.01 g	12.4 ± a	24.7 ± a	3.1 ± 0.1 a	1.11 ± 0.01 a	55.4 ± 0.1 e	8.09 ± 0.1 a	35.1 ± 0.8 b

n.s.: not significant; within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

Among the several chocolate appearance parameters, such as brightness, shape, surface smoothness and translucency, colour is one of the most impactful aspects on consumer choices [41]. The integration of *H. rhamnoides* fruit parts into chocolate caused a change in colour from the white of the untreated chocolate to darker tonalities, considering the orange-brown colour of the included materials, as shown by the decreased values of the L and b components and augmented b levels. Indeed, sea buckthorn material strongly influences the 'a' and 'b' colour components of chocolate because of its high pigment content [23], whereas the addition of other fruits was not found to significantly change the colour components of food products [9,47]. The addition of omega 3 fatty acids to chocolate elicited a darker [9] or redder colour [48] compared to the control, though the mentioned parameter also depends on ingredients and the manufacturing system [49]. Moreover, the fat bloom also has a significant effect on the Whiteness Index, i.e., the chocolate's visual and textural properties [9].

3.3. Antioxidant Compounds and Activity

The antioxidant compounds and activity (Table 6) showed the lowest values in the untreated chocolate, mainly referring to vitamin C, which was almost absent. The rising concentration of added *H. rhamnoides* material resulted in increased levels of all the antioxidants measured, as well as the antioxidant activity. Among the compounds examined, vitamin C showed the highest content, whereas the values of lycopene and flavonoids were the lowest. Regarding the different sea buckthorn materials, the juice addition to chocolate led to the highest contents of vitamin C, total carotenoids, β -carotene and lycopene, whereas the top levels of flavonoids, polyphenols and antioxidant activity were recorded upon the whole fruit integration. All the mentioned parameters attained the lowest contents in the *H. rhamnoides*-by-product-added chocolate.

In the latter respect, the high pigment content in sea buckthorn material added to chocolate influences the colour during the product processing [23].

The whole *H. rhamnoides* fruit has a greater polyphenol content compared to the structural constituents [23], thus influencing antioxidant compounds in chocolate.

Previous authors recorded the increase in total polyphenols and antioxidative capacity upon the integration of either grape seeds or pomace into chocolate [11,50,51], which enhanced the phenol content already present in cocoa particles. Moreover, grape pomace and seed powders can be adequately valorised as the prevailing polyphenol source following juice extraction [52]. Grape peels fostered both the polyphenol accumulation as well as darker colour and fruity taste in chocolate, compared to more neutral-tasting seeds. Notably, pomaces can be effectively replaced by purified extracts [50,53]. Interestingly, metabolizing phenolic compounds encouraged antioxidant activity in the plasma and urea of the tested individuals [54].

Table 6. Antioxidant characteristics of chocolate added with *H. rhamnoides* fruit materials.

Type of <i>Hippophae rhamnoides</i> Fruit Material (TP) × Percentage of Addition (PA)	Vitamin C (mg 100 g ⁻¹)	Flavonoids (mg CE g ⁻¹)	Polyphenols (mg GAE g ⁻¹)	Antioxidant Activity (μmol Trolox eq. g ⁻¹)	Total Carotenoids (mg g ⁻¹)	β-Carotene (mg g ⁻¹)	Lycopene (mg g ⁻¹)
Chocolate with no additions	0.1 ± 0.0 h	0.08 ± 0.02 h	0.19 ± 0.03 il	4.8 ± 0.2 i	0.10 ± 0.01 f	0.51 ± 0.03 g	0.04 ± 0.02 h
Chocolate with 10% juice addition	24.6 ± 1.0 f	0.15 ± 0.01 g	0.26 ± 0.01 h	7.0 ± 0.3 i	0.85 ± 0.10 e	0.68 ± 0.03 f	0.18 ± 0.01 e
Chocolate with 15% juice addition	28.2 ± 2.0 de	0.18 ± 0.01 f	0.32 ± 0.02 g	9.2 ± 0.5 h	1.23 ± 0.10 d	0.96 ± 0.05 e	0.19 ± 0.01 e
Chocolate with 20% juice addition	29.9 ± 1.0 d	0.20 ± 0.01 e	0.37 ± 0.02 f	15.3 ± 1.0 f	2.34 ± 0.10 b	1.81 ± 0.10 c	0.24 ± 0.02 c
Chocolate with 25% juice addition	42.2 ± 2.0 a	0.25 ± 0.01 d	0.44 ± 0.02 e	19.5 ± 1.0 d	2.61 ± 0.15 a	1.97 ± 0.10 b	0.34 ± 0.02 b
Chocolate with 10% fruit addition	21.1 ± 1.0 g	0.26 ± 0.01 d	0.48 ± 0.02 d	17.6 ± 1.0 e	0.73 ± 0.04 e	0.55 ± 0.03 g	0.15 ± 0.01 f
Chocolate with 15% fruit addition	28.2 ± 1.0 de	0.29 ± 0.01 c	0.52 ± 0.03 c	23.6 ± 1.0 c	1.21 ± 0.10 d	0.91 ± 0.05 e	0.16 ± 0.01 f
Chocolate with 20% fruit addition	33.4 ± 1.0 c	0.38 ± 0.02 b	0.65 ± 0.03 b	27.6 ± 1.5 b	1.74 ± 0.10 c	1.31 ± 0.07 d	0.21 ± 0.01 d
Chocolate with 25% fruit addition	37.0 ± 1.0 b	0.48 ± 0.02 a	0.84 ± 0.04 a	35.0 ± 1.5 a	2.75 ± 0.15 a	2.07 ± 0.10 a	0.39 ± 0.02 a
Chocolate with 10% by-product addition	21.1 ± 1.0 g	0.09 ± 0.01 h	0.17 ± 0.01 l	4.6 ± 0.2 i	0.13 ± 0.01 f	0.09 ± 0.01 i	0.12 ± 0.01 g
Chocolate with 15% by-product addition	24.6 ± 1.0 f	0.13 ± 0.01 g	0.21 ± 0.01 i	6.1 ± 0.3 il	0.13 ± 0.01 f	0.10 ± 0.01 i	0.12 ± 0.01 g
Chocolate with 20% by-product addition	26.4 ± 2.0 ef	0.14 ± 0.01 g	0.24 ± 0.01 h	10.5 ± 1.0 h	0.18 ± 0.01 f	0.13 ± 0.01 hi	0.13 ± 0.01 g
Chocolate with 25% by-product addition	29.9 ± 2.0 d	0.18 ± 0.01 f	0.31 ± 0.02 g	12.2 ± 0.6 g	0.24 ± 0.01 f	0.19 ± 0.01 h	0.15 ± 0.01 f

All data are expressed per fresh weight; n.s.: not significant; within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

Chocolate fortification with vitamin D and magnesium–calcium carbonate nanoparticles elicited a remarkable increase in polyphenol concentration in a previous investigation [10], whose level depends on the ratio between cocoa and hazelnut, the latter showing a high content of the mentioned antioxidant compounds [55]. Chocolate's high antioxidant content is beneficial to enhance flow-mediated dilatation, thus reducing cardiovascular disease risk in smokers [56]. Several factors, such as mechanical destruction, food matrix and processing, permanence in different gastrointestinal conditions and interactions with other diet constituents, influence the enzymatic hydrolyzation of polyphenol forms in food, such as esters, glycosides and polymers, prior to their absorption [57]. Notably, the reported bioaccessibility of polyphenols [58] triggers a changing trend of these compounds' content, with initial increase, subsequent reduction and final rise in the Simulated Salivary, Simulated Gastric and Simulated Intestinal phases, respectively [10]. Similarly, pH-significant reduction and subsequent increase were recorded in the stomach and neutral–basic intestine, respectively. Similar results were reported in previous research regarding vegetable juices [59] and fruits [60]. The bioaccessibility of grape polyphenols was higher in the Simulated Salivary phase than in the Simulated Gastric phase and Simulated Intestinal phase (50% vs. 20%) [61]. Moreover, polyphenol bioaccessibility decrease was caused by the interaction between milk caseins and black tea polyphenols, resulting in protein–polyphenol complexes characterized by hydrogen or hydrophobic bonds [62]. The matrix degradation enhancement is due to Simulated Gastric phase acidity and intestinal tract lipase activity, and the fortification process did not influence the in vitro digestibility of the derived products.

The addition of *Moringa oleifera* leaf extracts improved the nutritional and functional properties of white chocolate [63].

The addition of plant parts/extracts to chocolate enhances its health-beneficial properties, including bioactive compounds and nutrients [64,65]. In previous research [66], the integration of green tea extract in chocolate increased the total concentration of phenolic compounds and reduced the sugar content. Other research showed the high antioxidant value of peanut peel to valorise chocolate [67].

The addition of blueberry, blackberry, raspberry, and pomegranate juice significantly enhanced the phenolic content of white chocolate [68–70].

The addition of plant extracts of elderberry (*Sambucus nigra*) and, even more, chokeberry (*Aronia melanocarpa*) enhanced the antioxidant activity, moisture, fat content, viscosity and antimicrobial properties of chocolate [71,72].

Seaweed extracts, such as spirulina and kelp, were effective in fortifying chocolate, thus producing a functional food, due to their remarkable content of antioxidants, polysaccharides, nutrients, fatty acids, amino acids and vitamins [73,74].

Chocolate limited cadmium-induced toxicity in mice by reducing DNA damage, apoptosis, cell necrosis, oxidative stress and restoring mitochondrial function [75].

A recent investigation highlighted chocolate's effect on neurocognitive functions, i.e., modulating memory, attention and flexibility [76].

Chocolate has been shown to foster butyrate-producing bacteria growth, improving gut health [5].

The extracts of all the fruit components have been traditionally used for their antioxidant, anti-inflammatory and metabolism-regulating effects and for treating several diseases, among which are gastric, hepatic, dermatological, cardiovascular, cancer and neurological [77].

Due to the phenolic constituent action, the sea buckthorn fruit treatment reduced the concentration of carbonyl groups in plasma protein treated with H₂O₂ or H₂O₂/Fe and inhibited the rate of lipid peroxidation [78]. In addition, the mentioned application was shown to suppress cyclin expression, positively contrasting the proliferation of colon

cancer, and at the dose of 50 mg kg⁻¹, polyphenols significantly reduced tumour volume and growth in mice [79].

Effects of topical treatment with sea buckthorn fruit extract were recorded in previous research [80] on patients affected by mild to moderate psoriasis.

Treatment with sea buckthorn berry powder can cure Alzheimer's disease by removing intracellular Ab deposits connected with this neurodegenerative disorder, causing histopathological changes [81].

Sea buckthorn fruit fermentation solution exerts a hepatoprotective effect, regulating hepatic lipid metabolism and oxidative stress by modulating the composition of the intestinal microbiota [82], due to the carotenoids (b-carotene, lycopene, lutein, and b-cryptoxanthin) exhibiting hepatoprotective activity by reducing oxidative stress and regulating lipid metabolism in hepatocytes [83].

3.4. Mineral Elements

As for the mineral elements analyzed (Table 7), potassium and zinc contents were the highest in the untreated control upon the lowest addition of sea buckthorn-fruit-juice, and decreased with the rise in the *H. rhamnoides* material integration. As for the comparison between the sea buckthorn materials, the juice integration resulted in the highest values of K and Zn and the by-products in the lowest ones. Opposite trends to those described for potassium and zinc were shown by calcium, magnesium, sodium and phosphorus, which increased with the rising sea buckthorn material addition and attained the highest contents in by-product-integrated chocolate.

The mineral elements analyzed showed different trends as a response to the experimental treatments applied, which can be explained by the adsorption and cation exchange laws. Indeed, the retention of bivalent cations like Ca²⁺ and Mg²⁺ is stronger than that related to the monovalent cation K⁺ because of their smaller radius and lower hydration degree with consequent higher electrostatic field.

Based on the results obtained, compared to the untreated white chocolate, the addition of *Hippophae rhamnoides* fruit parts allows the ingestion of a lower product amount to reach the RDI (recommended daily intake) [84], depending on the mineral element: −21.6% for calcium, 26.6% for magnesium, 29.5% for phosphorus under the 25% by-product addition and 11.2% for sodium upon 25% juice addition (RDI equals to 1300, 420, 1250 and 2300 mg for Ca, Mg, P and Na, respectively). However, a very small additional amount of product is needed to reach the RDI for potassium and zinc (+3.2 and +2.0% for K and Zn, respectively, under the 10% juice addition, compared to the control).

Minerals are essential nutrients with different vital functions connected with important human metabolic activities and maintenance under the holistic and sustainable perspective [85,86]. In previous research [10], the addition of Mg Ca carbonate nanoparticles to chocolate led to a nine-fold and over two-fold higher content of calcium and magnesium, respectively, compared to other formulations. The latter differences in ingredients and their production patterns influence the mentioned variations [87]. Notably, the percentage of hazelnut can significantly affect the chocolate content in K, P, Ca, Mg, B, Cu and Mn [88]. Vitamin D integrated into chocolate was retained by over 90%, thus increasing its content up to almost 15 times.

Table 7. Mineral characteristics of chocolate added with *H. rhamnoides* fruit materials.

Type of <i>Hippophae rhamnoides</i> Fruit Material (TP) × Percentage of Addition (PA)	K (mg 100 g ^{−1})	Ca (mg 100 g ^{−1})	Mg (mg 100 g ^{−1})	Na (mg 100 g ^{−1})	P (mg 100 g ^{−1})	Zn (mg 100 g ^{−1})
Chocolate with no additions	86.7 ± 3.4 a	121.9 ± 5.1 e	51.7 ± 3.0 f	110.0 ± 5.8 bc	37.5 ± 2.1 g	1.56 ± 0.09
Chocolate with 10% juice addition	84.0 ± 4.4 a	132.1 ± 6.2 de	54.2 ± 3.3 ef	110.8 ± 5.3 bc	38.9 ± 2.0 fg	1.53 ± 0.09
Chocolate with 15% juice addition	77.3 ± 3.5 bc	136.1 ± 6.3 cd	58.1 ± 2.1 de	116.1 ± 5.3 ac	42.7 ± 2.4 df	1.50 ± 0.08
Chocolate with 20% juice addition	76.8 ± 3.9 bc	139.5 ± 6.3 bd	60.3 ± 3.5 cd	117.8 ± 5.7 ac	45.5 ± 2.4 cd	1.49 ± 0.08
Chocolate with 25% juice addition	71.8 ± 3.3 ce	144.3 ± 6.6 ad	63.5 ± 3.6 bd	123.9 ± 6.2 a	48.9 ± 2.5 bc	1.47 ± 0.08
Chocolate with 10% fruit addition	81.7 ± 3.3 ab	136.4 ± 6.1 cd	59.0 ± 2.9 de	107.2 ± 5.2 c	39.1 ± 2.1 fg	1.50 ± 0.08
Chocolate with 15% fruit addition	76.9 ± 3.9 bc	139.2 ± 5.9 bd	62.7 ± 3.5 bd	110.5 ± 5.6 bc	43.2 ± 2.2 df	1.48 ± 0.08
Chocolate with 20% fruit addition	73.8 ± 3.7 cd	144.5 ± 7.0 ad	65.6 ± 3.3 ac	112.7 ± 5.5 bc	47.9 ± 2.4 bc	1.44 ± 0.08
Chocolate with 25% fruit addition	66.7 ± 3.3 e	149.5 ± 6.5 ab	68.7 ± 3.3 ab	118.1 ± 5.6 ab	50.3 ± 2.7 ab	1.42 ± 0.08
Chocolate with 10% by-product addition	70.1 ± 4.1 de	140.9 ± 6.5 bd	61.3 ± 3.1 cd	107.4 ± 5.0 c	40.6 ± 2.2 eg	1.47 ± 0.08
Chocolate with 15% by-product addition	60.7 ± 3.6 f	145.3 ± 6.8 ac	65.2 ± 3.3 ac	111.5 ± 5.8 bc	43.7 ± 2.2 de	1.44 ± 0.08
Chocolate with 20% by-product addition	55.8 ± 3.1 f	150.0 ± 7.5 ab	68.2 ± 3.5 ab	114.7 ± 4.2 ac	48.0 ± 2.4 bc	1.42 ± 0.09
Chocolate with 25% by-product addition	49.6 ± 3.0 g	155.5 ± 7.4 a	70.4 ± 3.4 a	116.8 ± 5.8 ac	53.2 ± 2.7 a	1.40 ± 0.08

n.s.

n.s.: not significant; within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

3.5. Sensorial Features

Regarding the sensorial characteristics of chocolate (Figure 1 and Table 8), the untreated control showed the highest values of outer and inner colour, fineness of the additive particles, coherence, cocoa butter flavour and taste, animal fat flavour and taste, milk flavour and taste, chocolate flavour, breaking perception, general acceptance, satisfaction degree and recommendation, which, in some cases, are not significantly different from some applied treatments.

Upon increasing the *H. rhamnoides* fruit part addition, significant or tendential decreases were recorded of the inner aspect, outer and inner colour, fineness of the additive particles, taste, coherence, cocoa butter flavour and taste, animal fat flavour and taste, milk aroma and taste, chocolate flavour, intense sweet taste, mastication perception, general acceptance, satisfaction degree and recommendation. An opposite trend was shown by astringency, citrus and fruit flavour, sourness, bitterness, fruitiness, rancid and acidic taste, tastelessness, breaking perception, adhesiveness and hardness.

No significant differences were recorded for the outer aspect, bar thickness and probability of buying.



Figure 1. Sensorial features of chocolate added with *H. rhamnoides* fruit materials.

Table 8. Statistically different significances between the experimental treatments in terms of sensorial features of chocolate added with *H. rhamnoides* fruit materials.

TP × PA	AS	OC	IC	FP	T	A	CBF	AFF	MPF	ChF	CiF	FF	MPT	CBT	AFT	ST	BT	ST	FT	RT	AT	BT	TI	MP	BP	S	H	OI
UC	bd	a	a	a	ab	ad	a	a	a	a	e	c	a	a	a	f	a	bd	e	bc	d	e	ab	ab	a	bc	ac	a
C10JA	ad	ac	bd	b	bc	be	bc	c	bf	bd	be	ab	c	b	de	cd	b	cd	d	d	bd	bd	be	ac	c	c	ce	ad
C15JA	bd	bd	cd	bc	c	ac	bc	c	cf	cd	ad	ab	c	b	e	cd	b	cd	bc	d	ad	ac	ae	ad	bc	bc	bd	ae
C20JA	bd	cd	cd	bc	c	ab	cd	c	df	cd	ac	ab	c	bc	e	ab	b	cd	ac	cd	ad	a	ad	bd	bc	bc	ac	de
C25JA	ce	d	d	bc	c	ab	d	c	f	d	ac	a	c	c	e	ab	ab	d	ac	a	ac	a	ac	d	bc	ac	ac	e
C10FA	a	ac	bd	b	a	ce	a	ab	b	ab	ac	ab	b	b	bd	cd	bc	bc	bc	d	bd	ce	e	ab	bc	c	e	ac
C15FA	ab	ac	bd	bc	ac	ar	cd	c	cf	cd	ac	ab	c	b	be	bc	bc	bd	ac	cd	bd	ce	ac	bd	bc	bc	ac	ad
C20FA	ad	bd	bd	c	ac	ad	bc	c	df	cd	ab	ab	c	b	be	ab	ab	d	a	cd	ab	ce	ac	cd	bc	bc	ab	ad
C25FA	bd	cd	cd	c	bc	a	cd	c	ef	d	a	a	c	bc	ce	a	a	d	a	bd	a	ab	a	cd	ab	a	a	be
C10BPA	ab	ab	ab	a	ab	e	a	b	bc	ac	de	b	b	b	b	ef	d	a	cd	bd	d	e	e	a	c	ac	de	ab
C15BPA	ac	ac	ac	a	ab	e	a	b	bd	ad	de	ab	b	b	b	de	d	b	ac	bc	cd	e	e	ab	bc	ac	bd	ab
C20BPA	de	ac	bd	a	ab	de	ab	b	be	ad	de	ab	b	b	bc	cd	cd	bc	ac	b	bd	d	de	ac	bc	ab	ad	ad
C25BPA	e	bd	bd	a	ab	ce	ab	b	bf	bd	ce	ab	b	b	bd	cd	ab	bc	ab	b	ac	bd	ce	ac	bc	a	ad	ce

UC: Untreated control; C10JA: Chocolate with 10% juice addition; C15JA: 15% juice addition; C20JA: 20% juice addition; C25JA: 25% juice addition; C10FA: Chocolate with 10% fruit addition; C15FA: 15% fruit addition; C20FA: 20% fruit addition; C25FA: 25% fruit addition; C10BPA: Chocolate with 10% by-product addition; C15BPA: 15% by-product addition; C20BPA: 20% by-product addition; C25BPA: 25% by-product addition; Outer aspect: no significancy; AS: Aspect in section; OC: Outer colour; IC: Inner colour; FP: Fineness of *H. rhamnoides* particles; T: Taste; A: Astringency; CBF: Cocoa butter flavour; AFF: Animal fat flavour; MPF: Milk powder flavour; ChF: Chocolate flavour; CiF: Citrus flavour; FF: Fruit flavour; MPT: Milk powder taste; CBT: Cocoa butter taste; AFT: Animal fat taste; ST: Sour taste; BT: Bitter taste; ST: Sweet taste; RT: Rancid taste; AT: Acidic taste; BT: Bitter taste; TI: Tasteless; MP: Mastication perception; BP: Breaking perception; S: Stickiness; H: Hardness; OI: Overall impression. Within each column, mean values followed by different letters are significantly different at $p \leq 0.05$ according to Duncan test.

4. Conclusions

From research carried out on white chocolate, it was found that the addition of sea buckthorn (*Hippophae rhamnoides* L.) fruit, entirely or as juice or by-products, into chocolate at four concentrations (10%, 15%, 20%, and 25%), significantly affected the final product characteristics. Indeed, the chocolate was softer, browner and less sweet upon the sea buckthorn integration, with changes in protein and fat contents depending on the fruit part added. The addition of any *Hippophae rhamnoides* material type enhanced the antioxidant properties of white chocolate, thus showing the beneficial effects on the human organism with respect to the mentioned functional food. The positive outcome of the sea buckthorn fruit material addition to white chocolate obtained in the present research may have an interesting impact on the related industry to create a healthy, innovative product. Particularly, further investigations regarding the valorisation of *Hippophae rhamnoides* fruit by-product will be very useful from the environmental and economic points of view, from the perspective of the circular economy framework.

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