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Color Stability and Antioxidant Capacity of *Crataegus monogyna* Jacq. Berry Extract Influenced by Different Conditions

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Abstract: Hawthorn (*Crataegus monogyna*) berry is a horticultural product containing antioxidants and pigments. Its extract can be used as a food dye and antioxidant in food engineering. The aim of present study is to research the effects of different possible conditions (temperature, pH, interacting ions, and storage conditions) on the color and antioxidant capacity of hawthorn berry extract. Color was assessed by monitoring the CIELab parameters, while antioxidant capacity was measured using the reaction with ABTS and DPPH radicals. The total phenolic content in berry powder was 1146 mg GAE/100 g and the main polyphenols identified by HPLC were epicatechin, ferulic acid methyl ester, catechin, procyanidins B1 and B2, and various phenolic acids. The main carotenoids, also quantified by HPLC, were mutatoxanthin, lutein, α -cryptoxanthin, β -cryptoxanthin, cis- β -carotene, all-trans- β -carotene, and lycopene. The values of the overall color difference suggest that storage affected the color of the hawthorn extract more than any of the thermal treatments. Alkaline pH values affected color by changing the blue/yellow component, but also luminosity and the green/red parameter. The antioxidant capacity decreased in acidic and neutral media and increased in mildly alkaline media at pH 8.1. The possible presence of interacting salts as potassium nitrate and sodium chloride did not produce any significant changes in antioxidant capacity, while calcium chloride lowered it, but only at 0.001 M. The interaction with the studied salts had little effect on the extract's color. The obtained results demonstrated that hawthorn berry extracts can be used in the food industry as natural dyes, as it was proven to have very good antioxidant capacity and color stability after different thermal, pH, interacting salt, or storing conditions.

Keywords: hawthorn; CIELab color parameters; temperature; pH; ions; storage conditions



Citation: Cristea, E.; Ghendov-Mosanu, A.; Pintea, A.; Sturza, R.; Patras, A. Color Stability and Antioxidant Capacity of *Crataegus monogyna* Jacq. Berry Extract Influenced by Different Conditions. *Horticulturae* **2024**, *10*, 1184. <https://doi.org/10.3390/horticulturae10111184>

Academic Editor: Luigi De Bellis

Received: 21 September 2024

Revised: 4 November 2024

Accepted: 5 November 2024

Published: 9 November 2024



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1. Introduction

Hawthorn (*Crataegus monogyna*) is a small, rounded deciduous tree from the *Rosaceae* family with glossy, deeply lobed leaves and flat sprays of cream flowers, followed by dark red to deep orange berries in autumn [1], as their color can differ a lot depending on the variety. The ripe berries, which contain carbohydrates, organic acids, vitamins, saturated fatty acids, carotenoids, and various phenolics [2,3], can be used for juice fabrication, providing a refreshing and healthy beverage with a sour and tangy taste, and plenty of vitamins and antioxidants. Furthermore, these bioactive compounds also exist in byproducts resulting from the technological process of juice preparation.

In Europe, leaf and flower extracts of *Crataegus oxyacantha* or *Crataegus monogyna* have been used to treat mild to moderate congestive heart failure (NYHA I–III), and many clinical trials have shown that these preparations are beneficial in improving heart activity. Some studies have shown that hawthorn extracts have endothelium-dependent vasorelaxant

effects and inhibitory action on endothelin-1 [2], while others reported inhibitory activity on the human tumor cell growth of the phenolic extracts of flower buds and fruits [3].

Hawthorns also possess strong antioxidant properties [4]. While most studies focus on hawthorn's medicinal properties, its fruits may also have other uses, e.g., in sunscreens and cosmetic formulations due to their protective ability [5]. The extracts from hawthorn berries, but also their byproducts, can be used as natural food colorants due to their vibrant color, but also as antioxidants given their high antioxidant capacity. The pigments are mainly from the classes of polyphenolic compounds and carotenoids, both proving antioxidant capacity [6]. In general, flavonoids and procyanidins are the two main groups of active constituents in most varieties of hawthorn extracts, and in many state pharmacopoeias, these two groups are used to standardize and control the quality of hawthorn preparations [7]. Recent reports suggest that natural colors continue to gather speed in the shift towards natural ingredients and that their use is a basic requirement in functional foods formulation [8]. Thus, the use of natural pigments and antioxidants in food engineering can and should be expanded.

Data on the antioxidant capacity of the fruit extracts obtained from different Turkish *Crataegus* taxa suggest that hawthorn is one of the richest species in antioxidant compounds [9]. Several studies were undertaken to optimize the extraction of biologically active substances from various parts of the plant [9,10] and proved their antioxidant properties at cellular and mitochondrial level combined with high photostability [11].

Natural ingredients do tend to exhibit lower stability compared to their synthetic alternatives, which often represent either one or a simple mixture of compounds specifically developed to withstand harsh environments and thermal treatments. Therefore, model studies are necessary to identify optimal technological processes to be used when employing hawthorn extracts as either antioxidants or colorants.

This research provides new insights into the color stability of pigments from *Crataegus monogyna* var. *pendula* under various pH, temperature, and ion conditions, aiding in the development of valuable innovative natural food dyes. We also evaluated storage effects on antioxidant capacity and color. The hawthorn berries, sourced from southeastern Europe (Moldova), were analyzed for carotenoids; polyphenols, including flavonoids, flavonols, and cinnamic acids; antioxidant capacity; and color parameters. This study aims to elucidate some unknown aspects regarding the use of hawthorn as a natural pigments and antioxidants source, valorize this underused horticultural product, and encourage further research on replacing synthetic dyes with natural ones.

2. Materials and Methods

Crataegus monogyna var. *pendula* berries were harvested from plantations of Moldova (southeastern Europe geographical zone) in October 2022, when fully ripe. The trees' age was 9–11 years. The fruit's length was 8.21 ± 0.09 mm, and the width was 7.11 ± 0.13 mm. The mass of 100 fresh hawthorn fruits was 30.54 ± 0.23 g. Hawthorn trees in this area are resistant to drought and undemanding of soil.

2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) reagent, ferulic acid methyl ester, and sinapic acid were provided by Alfa Aesar (Pfalz, Germany); Folin–Ciocalteu reagent and acetonitrile by Merck (Darmstadt, Germany); α -cryptoxanthin, (+)-catechin, quercetin, syringic, ferulic, gallic, protocatechuic, para- and meta-hydroxybenzoic, p-coumaric, caffeic, and vanillic acids by Sigma (Darmstadt, Germany; Buchs, Switzerland); procyanidins B1/B2, hyperoside, β -carotene, lycopene, β -cryptoxanthin, and lutein were from Extrasynthese (Lyon, France); and resveratrol from TCI Europe (Zwijndrecht, Belgium). The purity of reagents varies between 95 and 99.9 %, being spectrophotometric, and, respectively, HPLC-grade, suitable for UV/Vis spectroscopy and HPLC. A Specord 200 Plus Analytic Jena (Jena, Germany) spectrophotometer with WinAspect Plus 4.2.0.0. software was employed for all spectrophotometric measurements.

2.1. Extraction

The hawthorn berries (Figure 1) were dried at 45 °C, chopped up to a yellow powder of 40 ± 10 , μm and sieved. The extraction was performed in 50% ethanol (1:10 ratio) under stirring for 30 min at room temperature (25 °C), according to a previously tested procedure [12], and resulted in a bright golden-yellow extract.



Figure 1. *Crataegus monogyna* var. *pendula* berries: fresh (a) and dry (b).

2.2. Studies on the Effect of Thermal Treatments and Storage Temperature

To study the effect of thermal treatments, the extract was submitted to different temperature–time regimes: -2 °C for 12 h; 4 °C for 24 h; 40 °C for 15 min; 60 °C for 15 min; 80 °C for 15 min; and 100 °C for 2 min. These thermal regimes were selected by considering the most widespread thermal technological treatments in the food industry. E.g., -2 °C is a typical frozen storage temperature; 4 °C—refrigeration; 40 °C—the typical temperature used for various fermentative and enzymatic treatments; 60 °C—the minimal temperature necessary to ensure the microbiological safety of foodstuffs; 80 °C—typical temperature used in classic thermal treatments such as pasteurization, HTST (high-temperature short time), PATS (pressure-assisted thermal sterilization); 100 °C—typical boiling and sterilization temperature. To study the effect of storage temperature, the extract was stored for two weeks at -2 °C, 4 °C, and ambient temperature (which fluctuated between 25 °C and 30 °C). After thermal treatments and storage, the antioxidant capacity and the CIELab color parameters of the extracts were assessed.

2.3. Studies on External Ion Influences

To study the influence of some ions that may exist in many food matrices on antioxidant capacity and color, salts of sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) were added to the extract. Sodium chloride, potassium nitrate, and calcium chloride, each at 3 concentrations, 0.001 M, 0.01 M, and 0.1 M, were introduced into the extracts. The antioxidant capacity and the color parameters (CIELab) were measured after a storage period of 12 h at 4 °C.

2.4. Studies on pH Influence

The initial native pH of the fresh extract is 5.6 and was adjusted to more acidic, less acidic, close-to-neutral, and mild alkaline values, i.e., 2.3; 3.9; 5.9; 7.6; and 8.1, which may be found in different foods. Various buffers were used to achieve this, as follows: for pH 2.3—3 mL buffer with pH 2.4 (prepared from 34.8 mL sodium citrate 0.1 M and 65.2 mL hydrochloric acid (HCl) 0.1 M per 100 mL solution), to which was added 1 mL HCl 0.1 M; for pH 3.9—4 mL buffer with pH 2.4 prepared as previously from sodium citrate and hydrochloric acid; for pH 5.9—0.5 mL 1/15 M phosphate buffer with pH 7.4 (prepared from 81.8 mL disodium hydrogen phosphate 1/15 M and 18.2 mL potassium dihydrogen phosphate 1/15 M per 100 mL solution); for pH 7.6—0.5 mL phosphate buffer prepared as previously, to which were added 0.2 mL sodium hydroxide (NaOH) 0.1 M; for pH 8.1—1 mL

phosphate buffer prepared as previously described, and 1 mL NaOH 0.1 M. The antioxidant capacity and the color parameters (CIELab) were analyzed for each tested pH.

The control samples for color parameters were prepared by diluting the extracts with the same volumes of 50% ethanol (the solvent used for extraction), matching the buffer volumes used for pH adjustments. Because the buffer solution was added in different amounts in case of each pH, each sample needed its own control. The controls were prepared to compensate the effects of dilution on color. In the case of antioxidant activity assessment [13], the control was the fresh extract (the effect of dilution being included in calculations). The adjusted extracts were stored at 4 °C for 12 h.

2.5. Antioxidant Capacity by Reaction with ABTS (2,2'-Azinobis [3-Ethylbenzothiazoline-6-sulfonic Acid]-Diammonium Salt) Radical

The antioxidant capacity of the samples was measured using the procedure described by Re et al. (1999) [13]. The results were expressed as mmol Trolox equivalents per 100 g hawthorn berry dry powder (mmol TE/100 g), from a calibration curve (0–2000 µmol/L) using Trolox as standard.

2.6. Antioxidant Capacity by Reaction with DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical

The stock solution of DPPH was prepared by diluting 0.0237 g of DPPH in ethanol 96%. For the preparation of the working solution, the stock solution was diluted with ethanol (96%) to the absorbance 0.6–0.7. To determine the antioxidant capacity, 3900 µL ethanolic solution of DPPH was taken into a tin-coated ampoule to minimize contact with light and 100 µL of sample was added. The mixture was intensely stirred and left in the dark for 30 min. The absorbance was measured at 517 nm. Ethanol 96% was used as reference. For the preparation of the control, 100 µL of ethanol (96%) was added to 3900 µL of DPPH solution. The results of antioxidant capacity were expressed in µmol Trolox equivalents per 100 g hawthorn berry dry powder (µmol TE/100 g DW) using a Trolox calibration curve (0–1500 µmol/L).

2.7. Folin–Ciocalteu Method for Total Polyphenols and Total Flavonoids

The total contents of polyphenols and total flavonoids were determined using Folin–Ciocalteu reagent, following the methods described by Ribereau-Gayon et al. (2006) [14] and Spranger et al. (2008) [15], respectively. The results were calculated from a calibration curve of gallic acid and expressed in mg equivalents of gallic acid reported to dry weight (mg GAE/100 g DW).

2.8. Total Polyphenols by Abs 280

Measuring the absorbance at 280 nm is another method employed to quantify the total polyphenol content. The result was expressed as milligrams equivalents of gallic acid reported to dry weight (mg GAE/100 g DW) from a calibration curve. The original procedure described by Ribereau-Gayon et al. (2006) [14] was followed.

2.9. Total Cinnamic Acids

The total cinnamic acids were quantified using the method described by Demir et al. (2014) [16]. The results were expressed as mg caffeic acid equivalents per 100 g dry powder (mg CAE/100 g DW) from a calibration curve (0–50 mg/L) with caffeic acid.

2.10. Total Flavonols

The total flavonols were determined following the method of Demir et al. (2014) [16]. The results were expressed as quercetin equivalents (QE/100 g DW) from a calibration curve (0–50 mg/L) of quercetin.

2.11. Total Content of Carotenoids

The content of total carotenoids was determined by the spectrophotometric method described by Biehler et al. (2010) [17]. For the analyzed samples, the maximum absorbance was determined at 450 nm.

2.12. The Content of Individual Carotenoids

For the quantification of individual carotenoids, the extraction was carried out with a mixture of methanol/ethyl acetate/petroleum ether (1:1:1, *v/v/v*). All extractions were performed in triplicate. After filtration, the residue was re-extracted twice using the same solvent mixture. Afterwards, the obtained extracts were saponified with 30% methanolic solution of potassium hydroxide (KOH) in the dark. To remove the soaps, the solution was washed several times with saturated sodium chloride solution and distilled water. The supernatant containing carotenoids was dried and evaporated to dryness using a rotary evaporator. Samples for analysis were dissolved in ethyl acetate and filtered through 0.45 mm polytetrafluoroethylene polymer (PTFE) filters. Reverse-phase high-performance liquid chromatography (RP-HPLC) analysis of carotenoids was performed on a Shimadzu LC-20AT with an SPD-M20A diode array detector (DAD) (Shimadzu Corporation, Kyoto, Japan). A YMC C30 column (250 × 4.6 mm; 5 µm) was used, and the mobile phases consisted of the following: solvent A—methanol/tert-butyl methyl ether/water (83:15:2); solvent B—methanol/tert-butyl methyl ether/water (8:90:2). The elution gradient was as follows: 0 min 0% solvent B, 20 min 0% B; 130 min –82% B; 132 min 0% B, followed by equilibration of column for 10 min. The flow rate was fixed at 0.8 mL/min and the DAD detector was set at 450 nm. The identification of carotenoids from the samples was carried out by the comparison of the ultraviolet–visible (UV–VIS) spectra and the retention times of the sample peaks with those of the standard solutions (Table 1).

Table 1. Retention time and wavelength of analyzed carotenoids.

R_t , min	Carotenoid	λ_{max} , nm
8.512	Mutatoxanthin	400, 427, 451
10.077	Lutein	421, 445, 473
34.829	α -cryptoxanthin	420, 445, 473
37.774	β -cryptoxanthin	428, 451, 476
70.451	cis- β -carotene	424, 446, 472
75.595	All-trans- β -carotene	421, 452, 478
99.325	Lycopene	448, 471, 503

2.13. Color Parameters (CIELab)

The following CIELab parameters were determined using the Specord 200 Plus (Jena, Germany) spectrophotometer: L^* —luminosity ranging from 0 (black) to 100 (white); a^* —green/red parameter (whose positive values indicate red color, and negative green); b^* —blue/yellow parameter (whose positive values indicate yellow nuances, and negative blue ones) [18]. The following parameters were calculated: C^* —chroma, a measure of color intensity/saturation ranging from 0 (completely unsaturated) to 100 or more (pure colour), $C^* = (a^{*2} + b^{*2})^{1/2}$; H^* —hue angle, reflecting the tone, $H^* = \arctan(b^*/a^*)$ and ΔE^* —overall color difference, reflecting the color change of the fresh extract after each treatment, $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [19]. The transmittance of the samples was measured every nm between 380 nm and 780 nm in an optical glass cuvette with a path length of 1 mm, using distilled water as reference, D65 as illuminant, and the observer placed at 10°.

2.14. Individual Polyphenols by HPLC

The content of individual phenolics was measured employing the Agilent 1100 Series HPLC (Santa Clara, CA, USA) following the method developed and described by Cristea et al. (2021) [20]. The following compounds were used as standards: gallic acid

($\lambda_{\max}$ = 280 nm; R_t = 5.294 min); protocatechuic acid ($\lambda_{\max}$ = 256 nm; R_t = 9.267 min); *p*-hydroxybenzoic acid ($\lambda_{\max}$ = 256 nm; R_t = 13.918 min); gentisic acid ($\lambda_{\max}$ = 324 nm; R_t = 15.531 min); procyanidin B1 ($\lambda_{\max}$ = 280 nm; R_t = 16.704 min); *m*-hydroxybenzoic acid ($\lambda_{\max}$ = 280 nm; R_t = 17.989 min); catechin ($\lambda_{\max}$ = 280 nm; R_t = 18.53 min); vanillic acid ($\lambda_{\max}$ = 256 nm; R_t = 20.319 min); caffeic acid ($\lambda_{\max}$ = 324 nm; R_t = 20.485 min); chlorogenic acid ($\lambda_{\max}$ = 324 nm; R_t = 22.871 min); procyanidin B2 ($\lambda_{\max}$ = 280 nm; R_t = 23.433 min); syringic acid ($\lambda_{\max}$ = 280 nm; R_t = 25.002 min); epicatechin ($\lambda_{\max}$ = 280 nm; R_t = 26.836 min); *p*-coumaric acid ($\lambda_{\max}$ = 324 nm; R_t = 29.695 min); ferulic acid ($\lambda_{\max}$ = 324 nm; R_t = 36.233 min); polydatin ($\lambda_{\max}$ = 280 nm; R_t = 38.234 min); sinapic acid ($\lambda_{\max}$ = 324 nm; R_t = 38.564 min); *trans*-resveratrol ($\lambda_{\max}$ = 324 nm; R_t = 49.333 min); *cis*-resveratrol ($\lambda_{\max}$ = 324 nm; R_t = 57.089 min); ferulic acid methyl ester ($\lambda_{\max}$ = 365 nm; R_t = 57.754 min); quercetin ($\lambda_{\max}$ = 256 nm; R_t = 65.278 min).

2.15. Mathematical Modelling

A first-order Sobol sensitivity analysis was performed using MATLAB (MathWorks, Inc., Natick, MA, USA) to investigate the effects of pH, temperature, and salt concentration on the chromatic parameters and antioxidant capacity. The Sobol sensitivity index, defined as the ratio of a factor's dispersion to the total output dispersion (1), was used to assess these influences.

$$\sum_i S_i + \sum_i \sum_{j>i} S_{ij} + \sum_i \sum_{j>i} \sum_{k>j} S_{ijk} + \dots = 1 \quad (1)$$

where for any input i , S_i constitutes the first-order Sobol sensitivity index and represents the sensitivity of the output to the changes of the input i ; indices with several arguments, such as S_{ij} , are for the second-order Sobol sensitivity index and represent the interaction between factors i and j ; those of the type S_{ijk} are for the third-order Sobol sensitivity index and represent the interaction between the factors i, j, k , etc. [21]. The higher the value of the first-order Sobol sensitivity index, the more pronounced the influence of the input factor on the output parameter.

2.16. Statistical Analysis

All statistical calculations were performed with IBM SPSS Statistics 23, considering the significance level $p \leq 0.05$. The results from three parallel experiments were used to calculate the means and the standard deviations. One-way and two-way ANOVA, as well as post-hoc Tukey test were applied for data differentiation and evaluation [12].

3. Results and Discussion

3.1. The Polyphenol Composition and the Antioxidant Capacity of Hawthorn Berries Extract

The content of total polyphenols (by two methods) and total carotenoids, the contents of different groups of polyphenols, the concentration of various individual phenolics and carotenoids (determined by HPLC), and antioxidant capacities are presented in Table 2.

Table 2. Composition and antioxidant capacity of the *Crataegus monogyna* var. *pendula* extract (the results are expressed as means $\pm$ standard deviations from three experiments and are reported to 100 g DW).

Parameter	Quantity, mg/100 g DW
Polyphenols	
Total polyphenols (Folin–Ciocalteu) GAE	1146 $\pm$ 230
Total polyphenols (Abs280) GAE	957 $\pm$ 71
Total flavonoids GAE	625 $\pm$ 40
Cinnamic acids CAE	388 $\pm$ 24
Flavonols QE	488 $\pm$ 23

Table 2. Cont.

Parameter	Quantity, mg/100 g DW
Epicatechin	8.1 ± 3.3
Ferulic acid methyl ester	6.7 ± 1.7
Catechin	4.2 ± 1.1
Procyanidin B1	2.2 ± 0.3
Ferulic acid	1.1 ± 0.2
Procyanidin B2	1.0 ± 0.2
Gallic acid	0.9 ± 0.2
<i>p</i> -hydroxybenzoic acid	0.9 ± 0.2
Vanillic acid	0.5 ± 0.3
Protocatechuic acid	0.4 ± 0.1
Syringic acid	0.4 ± 0.1
<i>p</i> -coumaric acid	0.3 ± 0.1
Sinapic acid	0.2 ± 0.1
Caffeic acid	0.2 ± 0.1
Hyperoside	Traces
<i>cis</i> -resveratrol	Traces
Quercetin	Traces
<i>m</i> -hydroxybenzoic acid	Traces
Carotenoids	
Total carotenoids	42 ± 2
Mutatoxanthin	0.062 ± 0.010
Lutein	0.064 ± 0.012
α -cryptoxanthin	0.045 ± 0.009
β -cryptoxanthin	0.046 ± 0.010
<i>cis</i> - β -carotene	0.028 ± 0.005
All- <i>trans</i> - β -carotene	0.043 ± 0.008
Lycopene	0.027 ± 0.006
Antioxidant capacity	
Antioxidant capacity (ABTS), mmol TE	7.54 ± 1.45
Antioxidant capacity (DPPH), mmol TE	2.025 ± 0.011

The extraction procedure employing 50% ethanol solvent led to important contents of polyphenols and antioxidant compounds, due to the solvent's polarity (determined by its high water content). This allowed for the polar phenolic compounds to be dissolved during the extraction process, resulting in increased extraction yields.

The contents of polyphenols determined by two methods were similar, i.e., 1146 mg GAE/100 g DW and 957 mg GAE/100 g DW when determined by Folin–Ciocalteu and by absorbance at 280 nm, respectively. Approximately 55% of the total polyphenols were flavonoids (Table 2). The main identified individual polyphenols were epicatechin, ferulic acid methyl ester, catechin, procyanidins B1 and B2, and many phenolic acids, among which were ferulic, gallic, *p*-hydroxybenzoic, vanillic, protocatechuic, syringic, *p*-coumaric, sinapic, and caffeic.

It has been documented that hawthorn berries contain epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, hyperoside, isoquercetin, and chlorogenic acid [22]. In the current experiment, catechin (4.2 mg/100 g DW), gallic acid (0.9 mg/100 g DW), procyanidin B1 (2.2 mg/100 g DW), and ferulic acid (1.1 mg/100 g DW) and its methyl ester (6.7 mg/100 g DW) were also identified.

Rodrigues et al. (2012) [3] characterized *C. monogyna* berries and identified flavonoids, flavones, and proanthocyanidins. Pawlaczyk-Graja (2018) [11] documented the existence of 49 distinct flavonoid compounds in extracts and the presence of the phenolic compounds rich in numerous free hydroxyl groups. Jarzycka et al. (2013) [5] studied the composition of hawthorn fruits from Poland and identified chlorogenic and neochlorogenic acids, *p*-coumaric acid glucoside, (-)epicatechin, *p*-coumaroyl quinic acid, rutin, hyperoside, quercetin-3-O-glucoside, rosmarinic acid, kaempferol-3-O-glucoside, and vitexin-200-O-

rhamnoside. Bardakci et al. (2019) [9] characterized the composition of polyphenols and the antioxidant capacity of the fruit extracts of several *Crataegus* taxa from Turkey. Their berries contained 391.97 ± 2.30 mg GAE/g total polyphenols, 17.60 ± 1.41 mg QE/g total flavonoids, 187.33 ± 7.07 mg CAE/g total phenolic acids, and the highest DPPH antioxidant capacity (1209.04 ± 4.61 expressed as EC₅₀ μ mol/g) among the species studied. Significant amounts of hyperoside and chlorogenic acid were mentioned [9]. The content of these bioactive compounds is influenced by factors like fruit freshness, preservation, extraction methods, cultivar, geography, soil, and climate.

Cosmulescu et al. (2017) [23] reported the presence of gallic acid, catechin, vanillic acid, chlorogenic acid, caffeic acid, syringic acid, epicatechin, coumaric acid, ferulic acid, sinapic acid, salicylic acid, rutin, ellagic acid, myricetin, *trans*-cinnamic acid, and quercetin (analyzed by HPLC-PDA) in hawthorn fruits from Romania—a country from the same geographical region and with similar climatic conditions comparing to the origin territory of the presently studied berries. The authors identified similar compounds and quantities in the Oltenia region of Romania, among which were gallic acid (0.82 mg/g fresh weight), catechin hydrate (3.03 mg/g fresh weight), vanillic acid (0.31 mg/g fresh weight), chlorogenic acid (3.21 mg/g fresh weight), caffeic acid (0.59 mg/g fresh weight), syringic acid (0.44 mg/g fresh weight), epicatechin (2.89 mg/g fresh weight), coumaric acid (0.24 mg/g fresh weight), ferulic acid (0.25 mg/g fresh weight), sinapic acid (1.77 mg/g fresh weight), salicylic acid (2.65 mg/g fresh weight), rutin (7.73 mg/g fresh weight), ellagic acid (7.56 mg/g fresh weight), myricetin (1.43 mg/g fresh weight), *trans*-cinnamic acid (0.19 mg/g fresh weight), and quercetin (5.72 mg/g fresh weight) [23]. Considering that moisture in hawthorn fruits is around 65% [24], the contents of some individual polyphenols are similar to those found in the current study, i.e., catechin, epicatechin, gallic acid, vanillic acid, ferulic acid, and sinapic acid.

The mentioned authors, who analyzed the polyphenol content and the antioxidant capacity of several different species, concluded that the antioxidant capacity determined in fruits was not directly affected by their total phenolics content, but that it was rather linked to the type of individual phenolic compounds.

Genotype is another factor to be considered, as reported by Garcia-Mateos et al. (2013) [25], who studied the phytochemicals and antioxidant capacity of the hawthorn species from Mexico. Li et al. (2020) [4] claim that phenolic compounds in hawthorn are the major bioactive molecules that contribute to its antioxidant capacity; nonetheless, carotenoids should also be considered.

Cui et al. (2006) [26] analyzed the concentration of seven polyphenols—epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, hyperoside, isoquercitrin, and chlorogenic acid—in the mature fruits of Chinese hawthorn (*Crataegus pinnatifida* Bge. var. major N.E.Br) using high-performance liquid chromatography and established that there was a significant inverse correlation between procyanidin content and the latitude of the geographical origin of the cultivars [26].

Li et al. (2020) [4] used ultra-performance liquid chromatography quadrupole-time of flight mass spectrometry to analyze free and bound polyphenols in raw hawthorn, identifying 26 soluble and 10 insoluble-bound compounds. They reported around 70 compounds, including flavonoids and triterpenoid acids. A positive correlation was found between polyphenol content and antioxidant capacity, notably with chlorogenic acid, procyanidin B2, hyperoside, and isoquercetin.

The above-described polyphenols along with carotenoids and other antioxidants result in relatively high antioxidant capacity, i.e., 7.54 mmol TE/100 g DW in the extract analyzed in this study. For comparison, Cosmulescu et al. (2017) [23] quantified the total antioxidant capacity of 70% methanol extract of *C. monogyna* fruits as 0.32 ± 0.01 mmol Trolox/100 g.

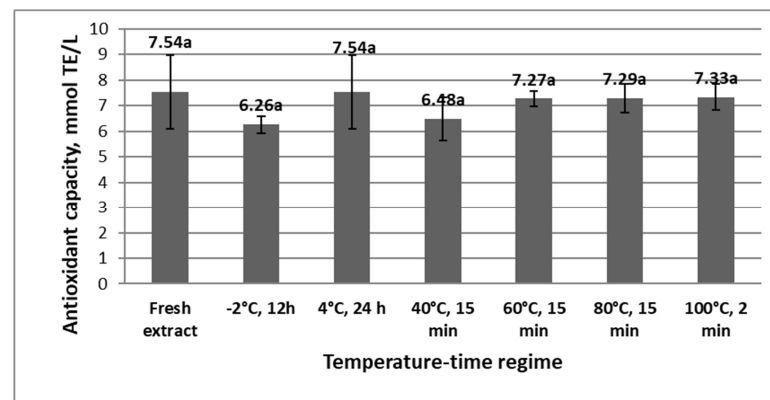
Lou et al. (2020) [27] determined the content of free, soluble esterified-bound, soluble glycosylated-bound, and insoluble-bound phenolic compounds, as well as the antioxidant capacity in Chinese hawthorn. The authors identified 22 major polyphenols using HPLC-ESI-MS/MS, among which (–)-epicatechin (750 ± 4 μ g/g DW) > procyanidin B2

($375 \pm 3 \mu\text{g/g DW}$) > (+)-catechin ($305 \pm 5 \mu\text{g/g DW}$) > chlorogenic acid ($256 \pm 4 \mu\text{g/g DW}$). There was a significant positive correlation between phenolic compounds and antioxidant capacity. Moreover, the antioxidant capacity of insoluble-bound phenolics in peel (103 ± 2 – $125 \pm 4 \mu\text{mol Trolox/g DW}$) was significantly higher than that in pulp (61.3 ± 1.3 – $67.3 \pm 1.4 \mu\text{mol Trolox/g DW}$) [27]. When recalculated in the same units, the values reported by the mentioned authors are very similar to the ones found in this study, i.e., $7.54 \text{ mmol TE/100 g DW}$.

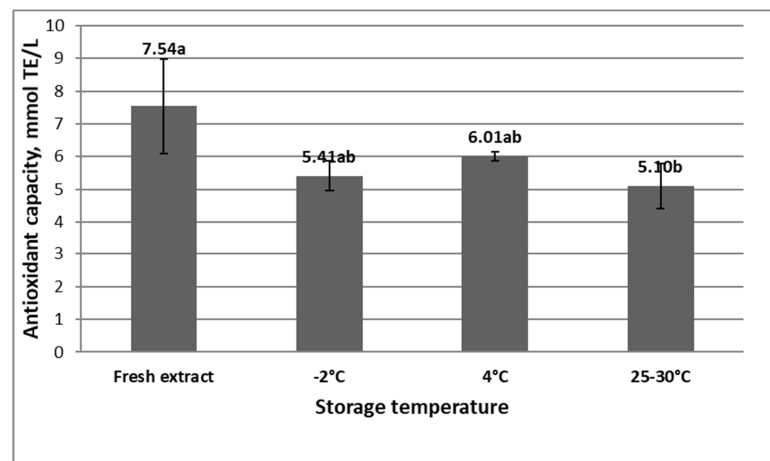
The total content of carotenoids in hawthorn can vary depending mainly on the species, the growing area, and the year of fruiting [28]. A total content of 42 mg/100 g DW was determined in our study, which correlates with data from the literature [29]. Lutein, mutaxanthin, α -cryptoxanthin, β -cryptoxanthin, *cis*- β -carotene, all-*trans*- β -carotene, and lycopene were identified and quantified. Their concentration was, however, relatively low. In berries, carotenoids are associated with proteins: carotenes and lycopene form complexes with proteins embedded in chromoplasts [30]. But processing operations, especially drying and grinding, reduce particle size, which favors the release of carotenoids. Lutein is micellarized to a greater extent than α -carotene and β -carotene, and xanthophylls (zeaxanthin, cryptoxanthin) have greater bioaccessibility due to hydroxyl groups that help increase their solubility in micellar structures [31].

3.2. Antioxidant Capacity (ABTS) of Hawthorn Berry Extract at Different pH, Ionic Strength, Temperature, and Storage Conditions

Figure 2 presents the antioxidant capacity (ABTS) of hawthorn berries extracts subjected to different thermal regimes (a), different storage temperatures (b), different pH values (c), and the presence of different salts (d).



(a)



(b)

Figure 2. Cont.

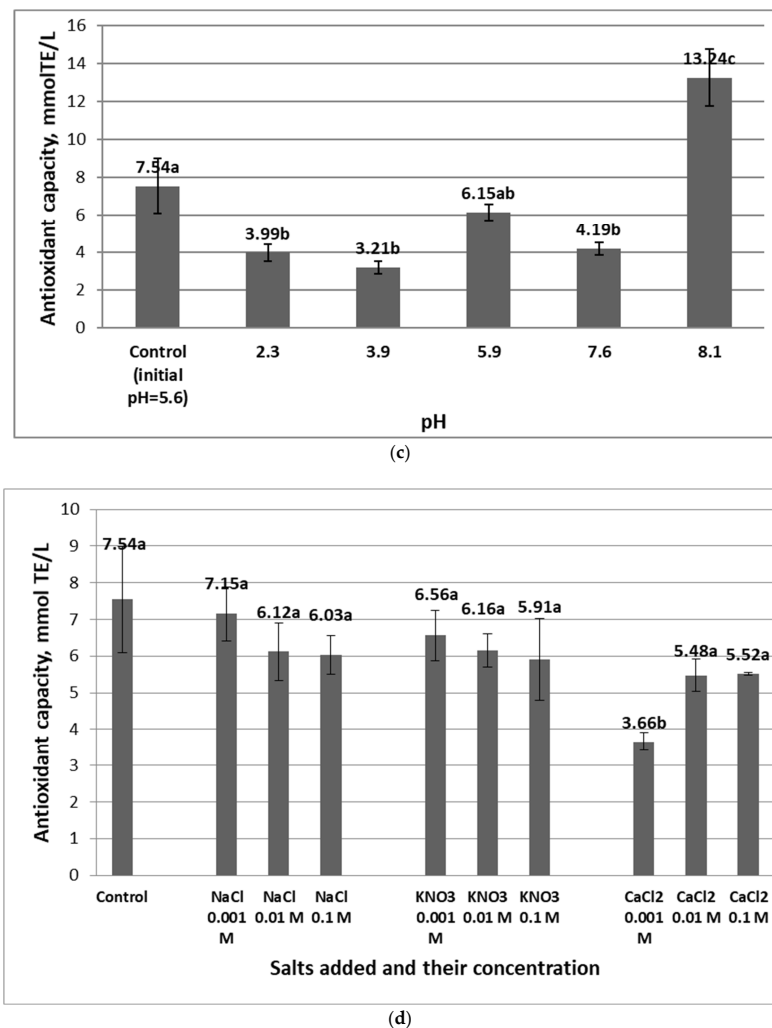


Figure 2. The antioxidant capacity (ABTS) of hawthorn berry extracts subjected to different conditions: different thermal regimes (a), different storage temperatures (b), different pH values (c), and presence of different salts (d). Different letters (a–c) designate statistically different results ($p \leq 0.05$).

The results for hawthorn extract submitted to different thermal regimes showed that none of the tested treatments significantly affected the antioxidant capacity. Thus, hawthorn extract is relatively stable at 4 °C for 24 h, when freezing for 12 h at −2 °C, when exposed for 15 min to 40 °C/60 °C/80 °C, or when exposed for 2 min at 100 °C (Figure 2a). This must be due to interactions with other compounds specific to the complex matrix of hawthorn berries, which were co-extracted under the conditions of the present study.

Li et al. (2020) [4] studied the stability of hawthorn berry extracts during thermal processing per the *Chinese Pharmacopoeia* (2010 edition), which was more aggressive than the present study's methods, and the matrix of the extract was different due to different extraction procedure/hawthorn cultivar/soil particularities/geographical and climatic conditions, etc. They found that thermal processing decreased soluble phenolics and increased total insoluble-bound phenolics. Procyanidin B2 and epicatechin significantly decreased and were undetectable after microwave treatment at 120 °C for 20 min and 150 °C for 15 min. Total antioxidant capacity decreased with higher heating temperatures. The impact on individual polyphenols varied, with some showing irregular changes while others remained stable due to glycosidic bonds.

Shortle et al. (2013) [10] recommend keeping the temperature between 35 °C and 60 °C when using supercritical fluid extraction with natural raw materials to avoid damaging thermolabile compounds.

All aforementioned studies indicate that temperature's impact on antioxidant capacity is less predictable due to factors like polyphenol state (free or bound), polyphenol type, fruit maturity, and surrounding medium, such as the food matrix, which can offer protection [4]. Our study proved that not all thermal treatments significantly change antioxidant capacity, as both freezing (12 h at $-2\text{ }^{\circ}\text{C}$) and heating (15 min at $40\text{ }^{\circ}\text{C}/60\text{ }^{\circ}\text{C}/80\text{ }^{\circ}\text{C}$, or 2 min at $100\text{ }^{\circ}\text{C}$) did not significantly affect hawthorn hydroethanolic extracts.

Figure 2b presents the changes in antioxidant capacity in hawthorn extracts stored for two weeks at different temperatures. The obtained values show that the antioxidant capacity decreased at all the investigated temperatures, but only the value determined in the extract stored at $25\text{--}30\text{ }^{\circ}\text{C}$ for two weeks proved to be statistically different from the control. The possible reason could be the oxidation, hydrolysis, or/and isomerization of polyphenolic compounds that occurs during storage.

Figure 2c shows the variations in antioxidant capacity in hawthorn extracts at different pH values. The more acidic pHs than the control sample, namely, 2.3 and 3.9, but also close-to-neutral (7.6), significantly decreased antioxidant capacity. Surprisingly, in the most alkaline pH (8.1), the antioxidant capacity increased almost twice, from 7.54 mmol TE/L to 13.24 mmol TE/L . A similar effect of decrease in neutral and acidic media and increase in alkaline media was previously observed in the case of other ethanolic (50%) extracts, i.e., chokeberry, rose hip, and grape marc [12,20,32].

In Figure 2d, the antioxidant capacity of extracts with different added salts are depicted. ANOVA analysis indicated no significant overall difference among the results, except for CaCl_2 at 0.001 M , which reduced antioxidant capacity. KNO_3 and NaCl additions showed no notable changes. Although other CaCl_2 concentrations also reduced antioxidant capacity, statistical tests deemed the changes insignificant. Further testing with a broader range of CaCl_2 concentrations and molecular studies to isolate the specific compounds are recommended to understand its effects fully. Previous studies have shown that calcium chloride decreased antioxidant capacity in various extracts such as grape marc, chokeberry, red cabbage, rose hip, and rowan berry [12,18,20,32]. Further studies are needed to elucidate the complex interaction mechanism that occurs in the presence of different interacting ions or at different pH.

3.3. CIELab Color Parameters of Hawthorn Berry Extracts at Different pH, Ionic Strength, Temperature, and Storage Conditions

Color was the main characteristic of the hawthorn extract studied in this research. Figure 3 presents the original fresh extract and the representation of its color in the CIELab space.



Figure 3. Original fresh extract (control) (a) and the representation of its color in the CIELab space (b).

The choice of heat treatments was made considering the manufacturing technologies of food products (confectionery and dairy products), in which hawthorn extract can be used as a natural dye (after solvent removal).

Table 3 presents the results of the color parameters for hawthorn extracts subjected to various temperatures for different periods of time (a) and stored for two weeks at different temperatures (b).

Table 3. The CIELab color parameters of hawthorn extract influenced by thermal treatments (a) and after 2 weeks of storage at 3 various temperature regimes (b) (the results are presented as mean $\pm$ standard deviation; different letters (^{a–d}) designate statistically different results; $p \leq 0.05$).

(a)						
Temperature-Time Regime	L*	a*	b*	C*	H*, °	ΔE^*
Fresh extract	92.25 $\pm$ 0.93 ^{ab}	−1.27 $\pm$ 0.13 ^{ab}	16.34 $\pm$ 0.33 ^{abc}	16.39 $\pm$ 0.32 ^{ab}	94.63 $\pm$ 0.00 ^a	—
−2 °C, 12 h	96.78 $\pm$ 0.30 ^b	−1.35 $\pm$ 0.02 ^a	14.46 $\pm$ 0.12 ^a	14.52 $\pm$ 0.12 ^a	95.20 $\pm$ 0.00 ^a	4.91 $\pm$ 0.67 ^a
4 °C, 24 h	95.31 $\pm$ 0.84 ^{ab}	−1.28 $\pm$ 0.12 ^{ab}	16.31 $\pm$ 0.28 ^{abc}	16.36 $\pm$ 0.27 ^{ab}	94.63 $\pm$ 0.57 ^a	3.06 $\pm$ 0.10 ^a
40 °C, 15 min.	96.81 $\pm$ 0.05 ^b	−1.39 $\pm$ 0.01 ^a	14.72 $\pm$ 0.20 ^{ab}	14.78 $\pm$ 0.20 ^a	95.20 $\pm$ 0.00 ^a	4.84 $\pm$ 0.90 ^a
60 °C, 15 min.	96.89 $\pm$ 0.06 ^b	−1.41 $\pm$ 0.01 ^a	15.06 $\pm$ 0.12 ^{ab}	15.13 $\pm$ 0.11 ^a	95.20 $\pm$ 0.00 ^a	4.81 $\pm$ 0.90 ^a
80 °C, 15 min.	94.32 $\pm$ 0.24 ^{ab}	−1.05 $\pm$ 0.03 ^{ab}	16.55 $\pm$ 0.14 ^{abcd}	16.58 $\pm$ 0.14 ^{ab}	93.48 $\pm$ 0.00 ^{ab}	2.09 $\pm$ 0.72 ^a
100 °C, 2 min.	92.91 $\pm$ 3.01 ^a	−0.79 $\pm$ 0.50 ^b	18.72 $\pm$ 2.38 ^{cd}	18.74 $\pm$ 2.36 ^{bc}	92.34 $\pm$ 1.72 ^b	2.52 $\pm$ 2.94 ^a
(b)						
Temperature and Storage Time	L*	a*	b*	C*	H*, °	ΔE^*
Fresh extract	92.25 $\pm$ 0.93 ^a	−1.27 $\pm$ 0.13 ^a	16.34 $\pm$ 0.33 ^a	16.39 $\pm$ 0.32 ^{ab}	94.63 $\pm$ 0.00 ^a	—
−2 °C, 2 weeks	96.62 $\pm$ 0.18 ^a	−1.38 $\pm$ 0.04 ^a	15.23 $\pm$ 0.21 ^a	15.29 $\pm$ 0.20 ^a	95.20 $\pm$ 0.00 ^a	17.10 $\pm$ 0.76 ^a
4 °C, 2 weeks	96.50 $\pm$ 0.01 ^a	−1.50 $\pm$ 0.02 ^a	17.10 $\pm$ 0.04 ^{ab}	16.39 $\pm$ 0.32 ^{ab}	95.20 $\pm$ 0.00 ^a	17.10 $\pm$ 0.97 ^a
25–30 °C, 2 weeks	95.07 $\pm$ 0.18 ^a	−1.05 $\pm$ 0.02 ^a	18.89 $\pm$ 0.10 ^b	18.92 $\pm$ 1.69 ^c	92.91 $\pm$ 0.00 ^b	20.76 $\pm$ 0.79 ^a

L*—luminosity; a*—green/red parameter; b*—blue/yellow parameter; C*—chromaticity; H*—hue angle; ΔE^* —overall color difference.

None of the investigated thermal treatments produced any significant change in luminosity (L*) compared to the control (fresh extract), and all values are close to 100, which represents the maximum lightness, assimilated to “white”. However, the extracts subjected to −2 °C for 12 h; 40 °C for 15 min; and 60 °C for 15 min had a significantly lighter color than the sample subjected to 100 °C for 2 min.

The same applies to the green/red component (a*), the blue/yellow component (b*), and to the chromaticity (C*); the values of these parameters were higher in the sample exposed to 100 °C for 2 min. Thus, this extract is slightly “less white”, slightly reddish, and yellower.

The hue angle, H*, has values over 90° in all cases (between 92.34° and 95.20°), holding positions in the second quadrant, in the yellow hue angle area. From all the samples, the yellower hue was obtained in case of the 100 °C, 2 min treatment (92.34°), and this was the only H* significantly different from the fresh extract.

The overall color difference, ΔE^* , showed small changes comparing to the overall color of the fresh extract, but the differences can be considered (theoretically) observable by the human eye, as it is generally stated that an observer can discriminate two colors if $\Delta E^* > 1$ [33]. In fact, because of different interferences, the color discrimination is usually worse [33]. All variations were less than 5 units (between 2.09 and 4.91) and differences among various treatments were statistically insignificant (Table 3a). The extract showed greater color stability after 2 min treatment at 100 °C when compared to similar extracts of rowan, sea buckthorn (unpublished data), and rose hip berries [20,34]. Overall, the thermal treatments globally preserve the initial color parameters of the hawthorn berry extract well, with slight differences that are not statistically significant.

Storage at each studied temperature, i.e., −2 °C, 4 °C, and 25–30 °C, produced an increase in luminosity (Table 3b). However, this change was not significant. Moreover, the green/red component (a*) remained almost unchanged, while the blue/yellow component (b*) slightly increased to yellower tones when stored at 25–30 °C. This change resulted in an improvement in color chroma, C*, which increases significantly during room temperature storage simultaneously with the significant decrease in H* angle towards a yellower hue. A

similar effect was also observed in the case of rose hip and rowan extracts when stored at room temperature [20,34].

The values of the overall color difference (ΔE^*) suggest that storage, regardless of the temperature, seriously affected the color of the hawthorn extract, more than any of the thermal treatments, with all values being higher than 17 units (between 17.10 and 20.76) compared to less than 5 units in case of thermal treatments (Table 3b compared to Table 3a). At the same time, regardless of temperature, the storage for 2 weeks of the extracts induced similar overall color differences, with statistically insignificant variations. Compared to other berry 50% hydroethanolic extracts, i.e., rowan and rose hip [20,34], hawthorn showed greater color stability during storage, regardless of temperature.

Table 4 presents the CIELab color parameters of the hawthorn extract after the addition of sodium chloride, potassium nitrate, and calcium chloride at three different concentrations.

Table 4. The influence of ionic strength on the CIELab color parameters of the hawthorn extract (the results are presented as mean $\pm$ standard deviation; different letters (^{a–c}) designate statistically different results; $p \leq 0.05$).

Salt and Concentration	L*	a*	b*	C*	H*, °	ΔE^*
Control	95.25 $\pm$ 0.93 ^a	−1.27 $\pm$ 0.13 ^a	16.33 $\pm$ 0.33 ^{bc}	16.38 $\pm$ 0.32 ^{bc}	94.63 $\pm$ 0.00 ^a	–
NaCl, 0.001 M	96.50 $\pm$ 0.05 ^a	−1.35 $\pm$ 0.01 ^a	14.92 $\pm$ 0.08 ^a	14.98 $\pm$ 0.08 ^a	95.20 $\pm$ 0.00 ^a	1.89 $\pm$ 0.92 ^a
NaCl, 0.01 M	96.49 $\pm$ 0.13 ^a	−1.34 $\pm$ 0.02 ^a	14.97 $\pm$ 0.08 ^a	15.03 $\pm$ 0.07 ^a	95.20 $\pm$ 0.00 ^a	1.84 $\pm$ 0.84 ^a
NaCl, 0.1 M	96.51 $\pm$ 0.12 ^a	−1.36 $\pm$ 0.02 ^a	14.83 $\pm$ 0.04 ^a	14.89 $\pm$ 0.04 ^a	95.20 $\pm$ 0.00 ^a	1.96 $\pm$ 0.87 ^a
KNO ₃ , 0.001 M	95.35 $\pm$ 0.17 ^a	−1.30 $\pm$ 0.01 ^a	17.39 $\pm$ 0.10 ^c	17.44 $\pm$ 0.10 ^c	90.62 $\pm$ 0.00 ^c	1.30 $\pm$ 0.60 ^a
KNO ₃ , 0.01 M	95.40 $\pm$ 0.28 ^a	−1.32 $\pm$ 0.06 ^a	17.42 $\pm$ 0.14 ^c	17.47 $\pm$ 0.14 ^c	94.06 $\pm$ 0.00 ^b	1.87 $\pm$ 0.67 ^a
KNO ₃ , 0.1 M	94.95 $\pm$ 0.37 ^a	−1.35 $\pm$ 0.16 ^a	17.55 $\pm$ 0.01 ^c	17.60 $\pm$ 0.01 ^c	94.06 $\pm$ 0.00 ^b	2.12 $\pm$ 0.11 ^a
CaCl ₂ , 0.001 M	95.91 $\pm$ 0.81 ^a	−1.30 $\pm$ 0.11 ^a	15.21 $\pm$ 0.92 ^b	15.27 $\pm$ 0.93 ^{ab}	94.63 $\pm$ 0.00 ^a	1.07 $\pm$ 0.80 ^a
CaCl ₂ , 0.01 M	94.96 $\pm$ 1.52 ^a	−1.28 $\pm$ 0.23 ^a	14.48 $\pm$ 0.64 ^a	14.53 $\pm$ 0.62 ^a	95.20 $\pm$ 1.15 ^a	1.10 $\pm$ 0.68 ^a
CaCl ₂ , 0.1 M	96.26 $\pm$ 1.04 ^a	−1.35 $\pm$ 0.16 ^a	14.47 $\pm$ 0.33 ^a	14.54 $\pm$ 0.32 ^a	95.20 $\pm$ 0.00 ^a	1.26 $\pm$ 0.65 ^a

L*—luminosity; a*—green/red parameter; b*—blue/yellow parameter; C*—chromaticity; H*—hue angle; ΔE^* —overall color difference.

None of the researched salts significantly affected luminosity (L*) and green/red parameter (a*). However, blue/yellow parameter (b*) was significantly decreased by more than one unit by NaCl (all concentrations) and CaCl₂ (0.01 M and 0.1 M), the yellow color component being diminished in these cases. At the same time, all KNO₃ treatments increased with about one unit the b* parameter. The changes in b* parameter induced variations in chroma (C*): significant decreases in the case of all NaCl concentrations and CaCl₂ 0.01 M and 0.1 M, as well as insignificant increases in case of all KNO₃ treatments. The H* angle remained the same for all NaCl and all CaCl₂ treatments, but significantly decreased for all KNO₃ treatments, and especially for 0.001 M, which was reflected in a yellower hue. Nonetheless, all the aforementioned particular modifications of the individual color parameters resulted in statistically similar overall color differences (ΔE^*) among tested salts (values between 1.07 and 2.12 units).

Table 5 presents the results of the CIELab parameters of hawthorn extract after pH adjustment compared to the control of each variant. The results obtained show that only the close-to-neutral (7.6) and alkaline pH (8.1) had a significant effect on the luminosity, by decreasing this parameter and darkening the extract. The green/red parameter (a*) was significantly affected by all tested pH and increased in all cases, especially at alkaline pH, which indicates a shift in color to redder tones. In the case of pH 5.9, 7.6, and 8.1, the green components of color (negative values of a*) were transformed in red components of color (positive values of a*), and the most important variation was registered in the case of pH 7.6.

Table 5. The influence of pH on the CIELab color parameters of hawthorn extract (the results are presented as mean $\pm$ standard deviation; different letters (^{a–d}) designate statistically different results; $p \leq 0.05$).

CIELab Parameters	L*	a*	b*	C*	H*, °	ΔE^*
Control for 2.3 pH = 2.3	98.4 $\pm$ 0.0 ^a 97.4 $\pm$ 0.2 ^a	−0.6 $\pm$ 0.0 ^a −0.3 $\pm$ 0.0 ^b	5.6 $\pm$ 0.1 ^a 6.5 $\pm$ 0.6 ^a	5.6 $\pm$ 0.1 ^a 6.5 $\pm$ 0.6 ^a	90.10 $\pm$ 0.00 ^a 92.34 $\pm$ 0.00 ^b	1.38 $\pm$ 0.54 ^a
Control for 3.9 pH = 3.9	99.1 $\pm$ 0.0 ^a 98.0 $\pm$ 0.2 ^a	−0.6 $\pm$ 0.0 ^a −0.5 $\pm$ 0.0 ^b	4.7 $\pm$ 0.0 ^a 5.5 $\pm$ 0.2 ^a	4.8 $\pm$ 0.0 ^a 5.5 $\pm$ 0.2 ^a	97.49 $\pm$ 0.00 ^a 95.20 $\pm$ 0.00 ^b	1.36 $\pm$ 0.10 ^a
Control for 5.9 pH = 5.9	96.9 $\pm$ 0.1 ^a 95.6 $\pm$ 0.1 ^a	−1.3 $\pm$ 0.0 ^a 0.8 $\pm$ 0.0 ^b	12.5 $\pm$ 0.2 ^a 13.6 $\pm$ 0.1 ^a	12.6 $\pm$ 0.2 ^a 13.6 $\pm$ 0.1 ^a	95.78 $\pm$ 0.00 ^a 93.48 $\pm$ 0.00 ^b	2.70 $\pm$ 0.10 ^b
Control for 7.6 pH = 7.6	96.9 $\pm$ 0.1 ^a 93.3 $\pm$ 1.8 ^b	−1.3 $\pm$ 0.0 ^a 1.4 $\pm$ 0.3 ^b	12.5 $\pm$ 0.2 ^a 23.8 $\pm$ 0.7 ^b	12.6 $\pm$ 0.2 ^a 23.8 $\pm$ 0.7 ^b	95.78 $\pm$ 0.00 ^a 93.48 $\pm$ 0.00 ^b	12.16 $\pm$ 1.80 ^c
Control for 8.1 pH = 8.1	97.7 $\pm$ 0.0 ^a 93.8 $\pm$ 0.7 ^b	−0.9 $\pm$ 0.0 ^a 1.1 $\pm$ 0.1 ^b	8.4 $\pm$ 0.0 ^a 23.8 $\pm$ 0.6 ^b	8.4 $\pm$ 0.0 ^a 23.8 $\pm$ 0.6 ^b	96.35 $\pm$ 0.00 ^a 92.91 $\pm$ 0.00 ^b	16.01 $\pm$ 0.93 ^d

L*—luminosity; a*—green/red parameter; b*—blue/yellow parameter; C*—chromaticity; H*—hue angle; ΔE^* —overall color difference.

The blue/yellow parameter as well as the luminosity were significantly affected only at pH 7.6 and 8.1. Thus, the color of the extract was shifted to much yellower shades, with the most important variation for pH 8.1. These augmentations of b* also increased the chroma, and the most important variation was again for pH 8.1. A similar effect of alkaline pH was observed in the case of rose hip, sea buckthorn, and rowan berry extracts, which nevertheless were slightly more stable [20,32].

The hue angle was in the second quadrant, in the yellow area, and had significant changes for all pH, but it increased only at 2.3, while at 3.9, 5.9, 7.6, and 8.1, it decreased, moving to an even yellower hue. The overall color difference due to the pH, ΔE^* , was between 1.36 and 16.01, with small variations in acidic media, an important change at close-to-neutral pH ($\Delta E^* = 12.16$), and a large variation of 16.01 units at the studied alkaline pH. After a 2-week storage period, which notably affected the color regardless of temperature, pH levels of 8.1 and 7.6 were the most important factors that influenced the color positively, resulting in increased saturation (C*), yellowness, redness, and reduced luminosity.

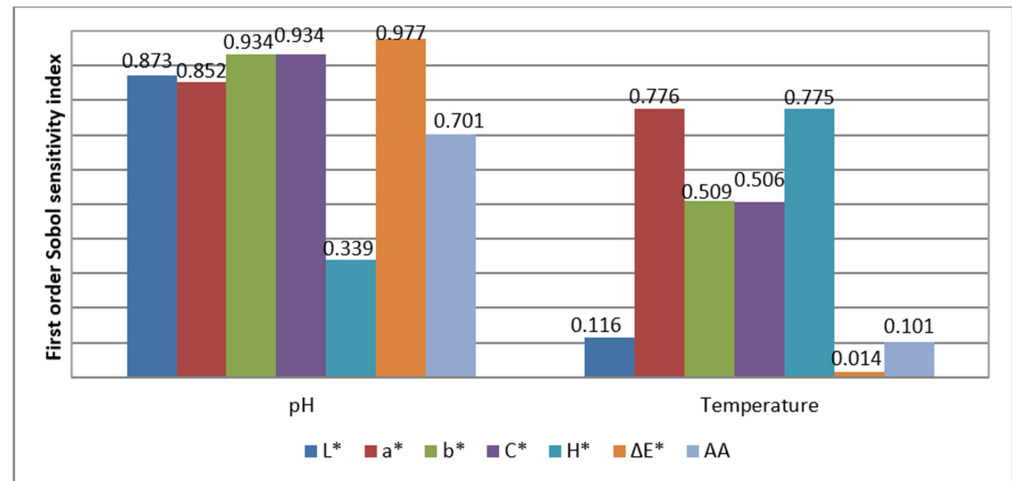
These findings are consistent with previously published data on carotenoids. Other authors report that carotenoids are prone to photooxidation and isomerization [35]. Exposure to light and the duration of the exposure are expected to be the main factors affecting their structure and properties. These molecules, especially lycopene, are less sensitive to heat, and can take a more stable cis isomer form past exposure to heat as long as the treatment duration is short and/or the temperature is kept under 100 °C. These cis isomers can also exhibit higher antioxidant activity [36]. Another study on carotenoid stability emphasized the critical role of pH, showing that carotenoids degrade faster in alkaline conditions (e.g., pH 8), while acidic environments help preserve them [37], which was confirmed by how color was affected by pH in this study. This highlights the importance of pH in maintaining the color and nutritional value of carotenoid-containing foods such as hawthorn berries. Future studies should focus on researching the correlation between the change in CIELab parameters and color assessment results obtained from a trained and specialized sensory panel, but also how consumers would perceive such changes, and whether they would impact their hedonic preferences.

3.4. Mathematical Modelling of the Results by First-Order Sobol Sensitivity Index

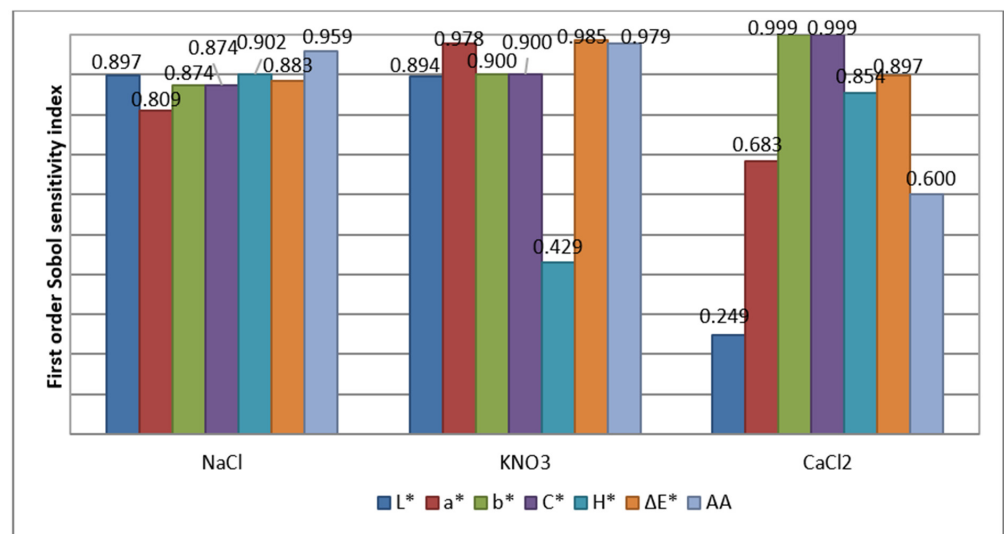
The Sobol method is a global sensitivity analysis that assesses the contribution of each input factor to the variance of the studied parameter. Sensitivity indices range from 0 to 1, as stated by Glen and Isaacs (2012) [38]. Information analysis allows for the evaluation of the mutual influences among determined parameters [39]. Based on two concepts (entropy and information), it uses the bit as unit of measure [40]. More certain predictions can be

made when the values of the mutual information are higher, and thus the uncertainties lower [41].

Figure 4a displays the sensitivity analysis using the first-order Sobol sensitivity index for pH and temperature effects on color parameters and antioxidant capacity in hawthorn extracts. A higher first-order Sobol sensitivity index value indicates a stronger influence of the input factor on CIELab parameters or antioxidant capacity.



(a)



(b)

Figure 4. Sensitivity analysis by the first-order Sobol sensitivity index: (a) influence of pH and temperature on chromatic parameters and antioxidant capacity in hawthorn extract; (b) influence of the concentration of different salts (NaCl, KNO₃, and CaCl₂) on chromatic parameters and antioxidant capacity. L*—luminosity; a*—green/red parameter; b*—blue/yellow parameter; C*—chromaticity; H*—hue angle; ΔE*—overall color difference; AA—antioxidant capacity.

Sobol index values show that pH had important influence on the overall color difference ΔE* (0.977), immediately followed by an equal influence on blue/yellow parameter b* and chromaticity C* (0.934), also luminosity L* (0.873), then green/red parameter a* (0.852) and antioxidant capacity AA (0.701). The hue angle H* was influenced by pH to a lesser extent (0.339).

In the case of temperature, all influences were much weaker, except the influence on the hue angle, which increased. The most important effects of the thermal treatments were

on the green/red parameter (0.776) and hue angle (0.775). Blue/yellow parameter (0.509) and chromaticity (0.506) were affected to a lesser extent. Overall color difference ΔE^* was the least influenced by temperature (0.014), while in the case of antioxidant capacity, the Sobol index was only 0.101 (Figure 4a).

Figure 4b shows the values of the first order Sobol sensitivity index concerning the influence of different salts concentration on the chromatic parameters and antioxidant capacity of hawthorn extract. In case of NaCl, the concentration strongly influenced all the analyzed parameters (values between 0.809 and 0.959): antioxidant capacity (0.959), followed by hue angle (0.902), luminosity (0.897), overall color difference ΔE^* (0.883), equally blue/yellow parameter and chroma (0.874), etc. KNO_3 concentration influenced to a high extent the overall color difference ΔE^* (0.985), antioxidant capacity (0.979), green/red parameter a^* (0.978), and equally blue/yellow parameter and chroma (0.9). Hue angle (0.429) was affected to the smallest extent. CaCl_2 concentrations exert the greatest influence on the blue/yellow parameter, as well as on chroma (0.999), followed by the overall color difference (0.897) and the hue angle (0.854). The value of the first-order Sobol sensitivity index for antioxidant capacity was 0.600. However, the smallest influence of CaCl_2 concentration was on the luminosity (0.249). As consequence, the Sobol sensitivity indices confirm the observations formulated during the results' interpretation and discussion.

4. Conclusions

Hawthorn (*Crataegus monogyna* var. *pendula*) berries originating from the Republic of Moldova (southeastern Europe) were characterized for the first time in terms of polyphenolic content and color parameters in order to use them as natural ingredients in food engineering.

The results proved a high content of total polyphenols (1146 mg GAE/100 g dry weight), from which 55% were flavonoids. The main individual polyphenols were epicatechin, ferulic acid methyl ester, catechin, procyanidins B1 and B2, and many phenolic acids, including ferulic, gallic, *p*-hydroxybenzoic, vanillic, protocatechuic, and syringic. The presence of a high content of polyphenols was reflected by a high antioxidant capacity.

The main carotenoids were mutatoxanthin, lutein, α -cryptoxanthin, β -cryptoxanthin, *cis*- β -carotene, all-*trans*- β -carotene, and lycopene.

To predict the behavior during/after various technological processes in the food industry, color parameters and antioxidant capacity in different conditions (temperature, pH, and interacting salts) were evaluated.

The results of the experiments involving thermal treatments suggest that *Crataegus monogyna* var. *pendula* extract has good thermal stability (keeping for 24 h at 4 °C, when freezing for 12 h at −2 °C, heating for 15 min at 40 °C/60 °C/80 °C, or heating for 2 min at 100 °C) when both color and antioxidant capacity are considered. Alkaline pH values significantly affected the color by changing mainly the blue/yellow component, but also the luminosity and the green/red parameter.

Antioxidant capacity increased significantly at pH 8.1 and decreased in acidic and close-to-neutral media. The addition of potassium nitrate, as well as sodium chloride, did not produce any statistically significant changes in the value of the antioxidant capacity, while calcium chloride lowered this parameter, but only when it was used at the lowest concentration of 0.001 M.

Interaction with salts had little effect on the color parameters. The values of the overall color difference (ΔE^*) suggest that storage for 2 weeks, regardless of the temperature, affected the color of the hawthorn extract more than any of the thermal treatments (ΔE^* being between 17 and 21), even if antioxidant activity was significantly decreased only by the 2 weeks' storage at 25–30 °C. However, the changes in color parameters did not lead to unpleasant colors; on the contrary, some of the chromatic characteristics were even improved (as chroma, C^*).

The present research strongly recommends *Crataegus monogyna* Jacq. var. *pendula* berries as natural and healthy replacements of the synthetic food dyes, as it was proven to

have very good antioxidant capacity and color stability after assessment under different thermal, pH, interacting salts, and storing conditions.

Author Contributions: Conceptualization, E.C. and R.S.; data curation, E.C. and A.G.-M.; formal analysis, E.C. and A.G.-M.; funding acquisition, A.P. (Antoanela Patras), A.P.(Adela Pintea), and R.S.; investigation, E.C.; methodology, E.C., A.G.-M., A.P. (Antoanela Patras), A.P. (Adela Pintea), and R.S.; coordinator, A.P. (Antoanela Patras), and R.S.; project administration, R.S. and A.P. (Antoanela Patras); resources, R.S., A.P. (Antoanela Patras), A.P. (Adela Pintea); software, E.C., A.P. (Adela Pintea) and A.G.-M.; supervision, A.P. (Antoanela Patras) and R.S.; validation, E.C., A.G.-M., A.P.(Adela Pintea), and A.P. (Antoanela Patras); visualization, E.C., A.P.(Adela Pintea), and A.G.-M.; writing—original draft—E.C.; writing—review and editing, A.P. (Antoanela Patras), A.G.-M., and R.S. All authors have read and agreed to the published version of the manuscript.

Funding: The APC was funded by the project AUF—DRECO-7865_SER-ECO_UTM_ENcap-LYPOSOM.

Data Availability Statement: The original contributions presented in this study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the Francophone University Agency (AUF), project AUF—DRECO-7863_SER-ECO_USVIIBI_DECHETJUS and project AUF—DRECO-7865_SER-ECO_UTM_ENcap-LYPOSOM, for funding this research; the “Ion Ionescu de la Brad” Iași University of Life Sciences (Romania) and the Technical University of Moldova (R. of Moldova) for research infrastructure; and also Marius Niculaua for providing technical expertise.

Conflicts of Interest: The authors declare no conflicts of interest.

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