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Assessment of the Antioxidant Potential of Blackthorns and Hawthorns: Comparative Analysis and Potential Use in Ruminants' Nutrition

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Abstract: The food industry is intensifying its effort to enrich food composition in various nutrients through animal feeding, but these challenges can be limited by the costly feed resources, water scarcity, and pesticide pollution, making it crucial to explore alternative feedstuffs with fewer requirements. Blackthorns and hawthorns are characterized by their rich phytochemical and antioxidant profiles, suggesting their potential to enhance the performance of ruminants though the supply of bioactive substances. Our study revealed their rich composition of micronutrients; hawthorns showed a remarkable amount of polyunsaturated fatty acids (57.23 g FAME/100 g total FAME), particularly omega-3 and omega-6, while blackthorn presented higher concentration of monounsaturated fatty acids, specifically oleic acid (56.99 g FAME/100 g total FAME). In terms of lipo-soluble antioxidants, blackthorn exhibited higher levels of xanthophyll and vitamin E (123.83 mg/kg DM), including its isomers (alpha, gamma, and delta). Concerning the water-soluble antioxidants, hawthorns showed elevated composition of the total content of flavonoids and polyphenols, comparing with blackthorn. Moreover, hawthorns showed a high antioxidant capacity, as assessed through DPPH, ABTS, and TAC analyses. In terms of the scavenging capacity of blackthorn and hawthorn against superoxide radicals, blackthorn had higher radical scavenging potential against superoxide radicals, compared to hawthorn.

Keywords: antioxidant capacity; bioactive compounds; blackthorn; hawthorn; ruminants

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1. Introduction

A balanced diet is the most essential aspect that can influence human's health status. Thus, considering that the world population is growing at an accelerating rate, this fact may represent a worry for the food industry. Animal nutrition is one of the most significant strategies used for naturally enriching animal food products with a variety of nutrient compounds; however, considering the limitation in feed resources, it is now more important than ever to value alternative feedstuffs [1,2].

Blackthorn (*Prunus spinosa* L.), originated from Rosaceae family, is spread mostly in uncultivated wild areas [3]. It represents a valuable source of antioxidant and antibacterial compounds, such as polyphenols, minerals, vitamins, flavonoids, etc. [4,5]. Furthermore, it exhibits an exceptional antioxidant capacity, with polyphenols being the main contributors of these results [6]. Various parts of blackthorn plant have been utilized for medicinal purposes, especially its fruits which demonstrated functional therapeutic properties. Traditionally, blackthorns can be employed in the treatment of hypertension, diabetes, and gastrointestinal disorders, and it can also be used in food product preparation (e.g., jams, liqueur) [7,8].

Hawthorn (*Crataegus* spp.) grows primarily in warm climates, being known for its toughness and strength due to its strong wood. Its chemical composition is rich in bioactive compounds, the most representative being polyphenols, especially phenolic acids, and flavonoids [9]. Therefore, hawthorns can exert impressive anti-hypertensive, anti-atherosclerosis, antibacterial, anti-inflammatory, and antioxidant properties [10]. Hawthorn is extensively utilized in traditional medicine and in the food and beverage industries [11]. In addition, hawthorn products are commonly recognized as natural solutions for promoting cardiovascular health [12].

The literature has highlighted the positive Impact of Incorporating natural antioxidants into ruminant diets on meat quality. Studies have shown that replacing barley grain with citrus pulp, a rich source of vitamin E, in lambs' diets increased the vitamin E content in meat [13] and improved the meat's antioxidant stability by reducing malondialdehyde content [14]. Furthermore, including pomegranate by-products, which contain antioxidant compounds, in lambs' diets resulted in decreased lipid oxidation in both fresh and cooked meat during refrigerated storage [15]. Regarding the effects of the dietary inclusion of blackthorns and hawthorns in ruminants' diet, the literature references are scarce, but several studies presented the important effects of the polyphenols on mitigating the inflammatory and oxidative state of the ruminants, which at a higher level can negatively impact the animals' performances and reproduction [16,17]. In addition, in vitro experiments demonstrated that polyphenols exert a significant effect on reducing methane emissions, which might be linked to both energy losses (which lowers the animals' ability to store and utilize energy) and environmental issues [18]. Also, it was demonstrated that polyphenolic compounds could enhance the quality of milk and meat [19].

Moreover, synthetic antioxidants are widely utilized in the nutrition of ruminants. However, consumers concerned regarding their safety and potential toxicity have encouraged the investigation into numerous natural alternatives, including plant sources [20].

Currently, blackthorns and hawthorns are not considered as feed ingredients, also because of their low level of main nutrients, such as protein. For example, they are not listed in the main feeding value tables., such as Feedipedia. However, their content in active substances raises the interest to valorize them as tools to manipulate rumen metabolism. In this perspective, the aim of this study was to nutritionally evaluate blackthorns and hawthorns as potential feed additives for ruminants' nutrition.

2. Materials and Methods

2.1. Plant Samples

The studied plants (whole plant of hawthorns and blackthorn fruits) were purchased from a local market (Bucharest, Romania) and analyzed in triplicates (n = 3).

Each sample of plants was dried to a constant weight, and grounded into a fine powder. Plants' samples were stored at room temperature and in the dark until analysis.

2.2. Analytical Standards

To determine vitamin E isomers, analytical standards from Sigma-Aldrich (St. Louis, MO, USA) were used: δ-tocopherol (95%), γ-tocopherol (96%), and α-tocopherol (96%).

For the determination of xanthophylls, the standards purchased from Sigma-Aldrich included lutein (95%), zeaxanthin (95%), and astaxanthin (97%).

The determination of fatty acids profile (FAME) was performed using a standard mixture from Merck (Darmstadt, Germany).

The phenolic standards were sourced from Sigma-Aldrich (St. Louis, MO, USA), including gallic acid (95%), epigallocatechin (96%), epicatechin (96%), ellagic acid (95%), 4-hydroxy-3-methoxycinnamic acid (95%), rutin (95%), syringic acid (98%), vanillic acid (95%), protocatechuic acid (96%), 3-hydroxybenzoic acid (95%), caffeic acid (95%), catechin (95%), quercetin (95%), resveratrol (99%), trans cinnamic acid (99%), and p-coumaric acid (98%). Ferulic (97%) and chlorogenic acids (95%) were obtained from the European Pharmacopeia (EP).

2.3. Proximate Chemical Composition

The assessment of the crude protein concentration for the blackthorns and hawthorns was performed using Kjeldahl method (ISO 5983-2/2009) [21], with Kjeltec auto 1030 Tecator Instruments (Höganäs, Sweden). The crude fat content was obtained using Soxhlet method (SR ISO 6492/2001) [22] by a Soxtec 2055 Foss Tecator, (Höganäs, Sweden). The determination of the crude fiber content was performed by intermediary filtration using a Fibertec 2010 System Foss Tecator, (Höganäs, Sweden). The total ash content for the studied plants was obtained using a gravimetric method and a Nabertherm furnace (Nabertherm GmbH, Lilienthal, Germany) [23].

2.4. Minerals Composition

The minerals composition (manganese, copper, zinc, and iron) was measured using flame atomic absorption spectrometry (FAAS), following the microwave digestion, with a Thermo Electron SOLAAR M6 Dual Zeeman Comfort System (Cambridge, UK), as reported by [24].

2.5. Fatty Acids Profile

The fatty acids composition of blackthorn and hawthorn was obtained using gas-chromatography with a Perkin Elmer Clarus 500 chromatograph (Waltham, MA, USA), following the method described by [25]. For determination, the fatty acids were separated using a TRACE TR-Fame capillary column and filled with a high polar material (Thermo Electron, Waltham, MA, USA), with dimensions: 60 m × 0.25 mm × 0.25 µm film. The detection of the compounds was obtained using an FID detector. Before separation, an esterification reaction was performed on the fatty acids, and then they were converted into methyl esters. Their quantification was performed using the standard mixture.

2.6. Lipo-Soluble Antioxidant Compounds

The lipo-soluble antioxidant compounds were extracted from blackthorn and hawthorn using the method presented by [26]. Before analysis, a saponification step was required using a hydrolysis mixture consisting of a potassium hydroxide solution dissolved in ethanol. The samples were hydrolyzed at 80 °C for 30 min, followed by petroleum ether extraction.

The xanthophyll content (lutein, zeaxanthin, astaxanthin) was determined using a high-performance liquid chromatography (HPLC) method, with a Perkin Elmer 200 series System (Shelton, CT, USA), and a UV detector (λ = 450 nm) as described by [26]. The mobile phase consisted of acetone (75%), methanol (15%), and water (10%), and the separation was performed with a C18 reversed-phase column (Nucleodur, Macherey-Nagel, Germany), with dimensions: 5 µm, 250 mm × 4.60 mm interior diameter.

The determination of the vitamin E isomers was assessed using the HPLC method described by [26] with a Vanquish Core HPLC System (Thermo-Electron Corporation, Waltham, MA, USA), a PDA–UV detector (λ = 292 nm), and an Accucore C18 column (Thermo-Electron Corporation, Waltham, MA, USA) with the following dimensions: 4 µm particle size, 150mm × 4.6 mm. The mobile phase consisted of methanol (96%) and water (4%).

2.7. Water-Soluble Antioxidant Compounds

The total content of polyphenols (TP) was studied using a Folin–Ciocâlteu method, as reported by [23]. The results were presented as mg GAE /g–mg gallic acid equivalents/gram of dried samples, using the gallic acid calibration curve.

The total content of flavonoids (TF) was determined using a colorimetric method, involving aluminum chloride [26]. One mL of methanolic extract of the studied plants was mixed with aluminum chloride (4 mL), following the incubation for 15 min at room temperature. After incubation was completed, an orange–yellow solution was obtained, whose absorbances were read at 410 nm with a Jasco V-530 UV-VIS spectrophotometer

(Japan Servo Co., Ltd., Tokyo, Japan). The results were expressed as mg Que eq./g–mg quercetin equivalents/gram of dried samples.

Polyphenol profile was determined using a HPLC method [23], with a Vanquish Core HPLC System (Thermo Fisher Scientific, Waltham, MA, USA), a BDS HyperSil C18 column (Thermo Fisher Scientific, Waltham, MA, USA), with the following dimensions: 250 mm × 4 mm, 5 µm particle size, and DAD. The chromatographic method used three solvents for the mobile phase: A—acetic acid (1%); B—methanol, and C—acetonitrile. The elution of the mobile phase Involved a binary gradient as follows: 90% A, 5% B, 5% C (0–15 min); 81% A, 4% B, 15% C (15–20 min); 72% A, 3% B, 25% C (20–25 min); 60% A, 2% B, 38% C (25–40 min); 90% A, 5% B, 5% C (40–50 min).

2.8. Antioxidant Potential of the Studied Plants

The antioxidant capacity of the blackthorn and hawthorn was studied using three different methods, ABTS, DPPH, and total antioxidant capacity (TAC).

The ability of the studied plants to scavenge the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) was evaluated following the method described by [23], with a Jasco V-530 spectrophotometer (Japan Servo Co., Ltd., Japan) to measure absorbances at 734 nm.

The determination of the antioxidant capacity by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate method (DPPH) was obtained with a Jasco V-530 spectrophotometer (Japan Servo Co., Ltd., Japan), and the results were expressed as mmol Trolox equivalents per kg of sample [23].

The total antioxidant capacity (TC) was assessed using the phosphomolybdenum method, with absorbances measured at 695 nm. Results were expressed as equivalents of ascorbic acid [23].

The assessment of superoxide radical scavenging activity (SRSA) in blackthorn and hawthorn was performed following the method described by [26]. The absorbance was measured at 560 nm using a spectrophotometer (Jasco V-530, Japan Servo Co., Ltd., Tokyo, Japan). The inhibition (%) of the superoxide anion generation was calculated using the formula:

$$\% \text{ Inhibition} = (AC—AS) \times 100/AC$$

where AC—control absorbance (ascorbic acid); AS—sample absorbance.

2.9. Statistical Analysis

The statistical analysis was performed using XLSTAT software, 19.01 version (Addinsoft, Paris, France). The differences among mean values were considered statistically significant for p values < 0.05. The graphs were obtained using Prism-GraphPad software, version 10.2 (San Diego, CA, USA). Pearson's correlation coefficient test between the fatty acids' composition (SFA, PUFA, MUFA, omega 6, and omega 3 fatty acids), antioxidant compounds (total polyphenols, total flavonoids, isomers of vitamin E, and xanthophyll), and antioxidant potential (total antioxidant capacity, DPPH, ABTS, and the scavenging potential against superoxide radicals) for the blackthorn and hawthorn was presented as correlation heatmap. Purple colors correspond to positive correlation coefficients, and yellow colors correspond to negative correlation coefficients.

3. Results

3.1. Proximate Composition and Trace Mineral Content

The analysis of the proximate chemical composition revealed that blackthorns presented a significantly higher concentration of crude protein and crude fat, compared with hawthorns ($p = 0.003$, $p < 0.0001$) (Table 1). The higher concentration of the crude fiber was observed in case of hawthorns, while the ash content did not differ among the studied plants.

Table 1. Proximate composition of blackthorn and hawthorn (n = 3).

Parameters (%)	Blackthorns	Hawthorns	SEM [1]	p
Crude protein	3.54 [a]	3.25 [b]	0.031	0.003
Crude fat	2.875 [a]	2.01 [b]	0.023	<0.0001
Crude fiber	12.48 [b]	36.31 [a]	0.123	<0.0001
Ash	2.97	2.90	0.045	0.330

[1] The standard error of the mean, [a,b] means within a row with no common superscript differs ($p < 0.05$).

The evaluation of the trace minerals showed that hawthorns contained the higher levels of iron and zinc compared with blackthorns. In contrast, blackthorns displayed a greater concentration of manganese (Figure 1).

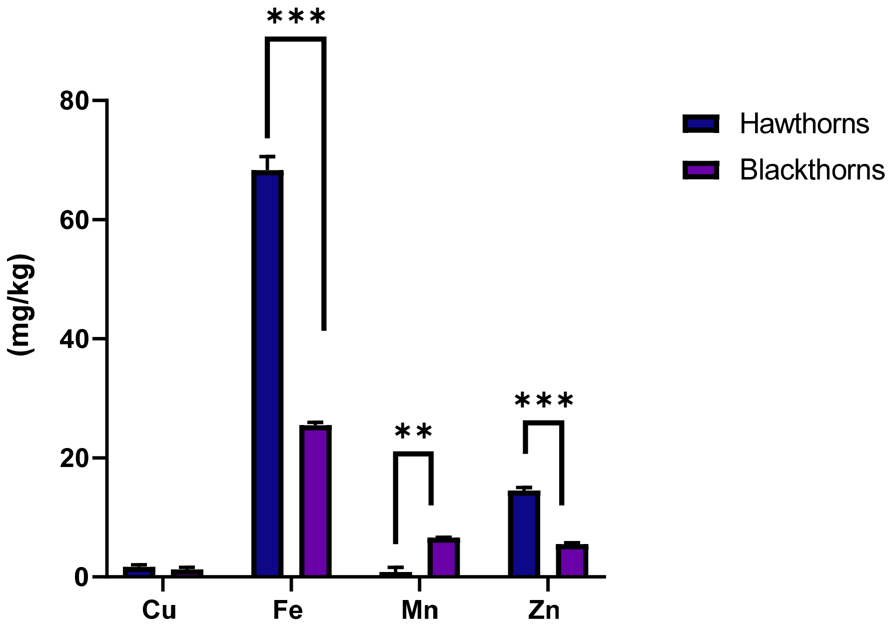


Figure 1. Trace minerals composition (copper, iron, manganese, zinc) of blackthorn and hawthorn. The results are presented as means ± SEM; **, *** represents significant differences between means (** $p \leq 0.01$, *** $p \leq 0.001$).

3.2. Fatty Acids Composition

The data regarding the fatty acids profile of the studied plants are presented in Table 2.

Table 2. Fatty acids profile of blackthorn and hawthorn (n = 3).

G FAME/100 g FAME		Blackthorns	Hawthorns	SEM [5]	p
Caproic acid	C6:0	Nd. [1]	0.097	-	-
Caprylic acid	C8:0	Nd. [1]	0.104	-	-
Capric acid	C10:0	Nd. [1]	0.411	-	-
Lauric acid	C12:0	Nd. [1]	0.065	-	-
Miristic acid	C14:0	0.134 [b]	0.721 [a]	0.008	<0.0001
Pentadecanoic acid	C15:0	Nd.	0.228	-	-
Pentadecenoic acid	C15:1	Nd. [1]	0.071	-	-
Palmitic acid	C16:0	8.911 [b]	10.151 [a]	0.007	<0.0001
Palmitoleic acid	C16:1	1.247 [a]	0.449 [b]	0.022	<0.0001
Heptadecanoic acid	C17:0	Nd. [1]	0.046	-	-
Stearic acid	C18:0	2.655 [a]	2.098 [b]	0.022	<0.0001
Oleic acid	C18:1	56.995 [a]	28.162 [b]	0.036	<0.0001
Cis-Linoleic acid	C18:2n6	28.229 [b]	53.715 [a]	0.017	<0.0001
Alpha-linolenic acid	C18:3n3	1.814 [b]	2.304 [a]	0.013	<0.0001
Octadecatetraenoic acid	C18:4n3	Nd. [1]	0.903	-	-
Eicosadienoic acid	C20:2n6	Nd. [1]	0.312	-	-

Table 2. *Cont.*

G FAME/100 g FAME	Blackthorns	Hawthorns	SEM [5]	*p*
Other fatty acids	0.014 [b]	0.164 [a]	0.023	0.010
ΣSFA [2]	11.700 [b]	13.921 [a]	0.018	<0.0001
ΣMUFA [3]	58.242 [b]	28.682 [a]	0.032	<0.0001
ΣPUFA [4]	30.043 [b]	57.233 [a]	0.021	<0.0001
Omega 3	1.814 [b]	3.222 [a]	0.010	<0.0001
Omega 6	28.229 [b]	54.026 [a]	0.015	<0.0001
Omega 6/Omega 3	15.560 [b]	16.768 [a]	0.015	<0.0001

[1] not determined, [2] sum of the saturated fatty acids, [3] sum of the mono-unsaturated fatty acids, [4] sum of the poly-unsaturated fatty acids, [5] the standard error of the mean, [a,b] means within a row with no common superscript differs ($p < 0.05$).

In our study, sixteen individual fatty acids were detected in hawthorn, whereas seven individual fatty acids were identified in blackthorn. The major fatty acids quantified in these plants were oleic acid and cis-linoleic acid (CLA). Hawthorns exhibited the highest concentration of polyunsaturated fatty acids (PUFAs) and a greater content of omega-3 and omega-6 fatty acids compared to blackthorns. Also, the obtained data showed that hawthorns had significantly higher concentrations of CLA and alpha-linolenic acid, and also contained higher levels of medium-chain fatty acids (MCFAs), ranging from C6 to C12. Regarding monounsaturated fatty acids (MUFAs), blackthorn presented the highest level of oleic acid.

3.3. Compounds with Antioxidant Activity

The contents of the lipo-soluble antioxidant compounds are presented in Table 3.

Table 3. Lipo-soluble antioxidant compounds (n = 3).

Parameters (mg/kg)	Blackthorns	Hawthorns	SEM [2]	*p*
Alpha-tocopherol	97.79 [a]	51.77 [b]	1.133	<0.0001
Gama-tocopherol	18.00 [a]	9.09 [b]	0.205	<0.0001
Delta-tocopherol	8.03 [a]	3.90 [b]	0.210	<0.0001
Total vitamin E	123.83 [a]	64.76 [b]	1.332	<0.0001
Lutein and zeaxanthin	123.09 [a]	52.29 [b]	3.179	<0.0001
Astaxanthin	Nd. [1]	0.105	-	-

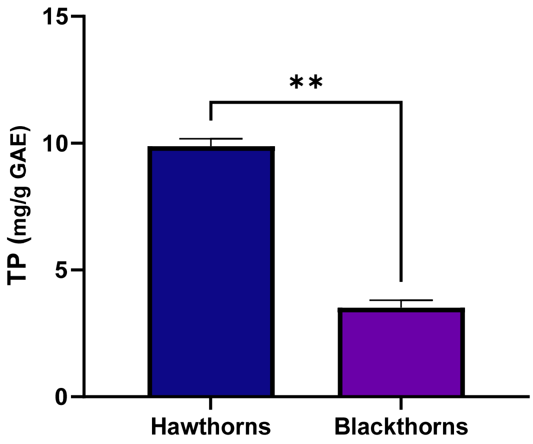
[1] not determined, [2] the standard error of the mean, [a,b] means within a row with no common superscript differs ($p < 0.0001$).

The results showed that the most abundant vitamin E isomer in both studied plants was the alpha-tocopherol, followed by gamma-tocopherol. In case of xanthophyll, lutein and zeaxanthin were the major compounds in both plants.

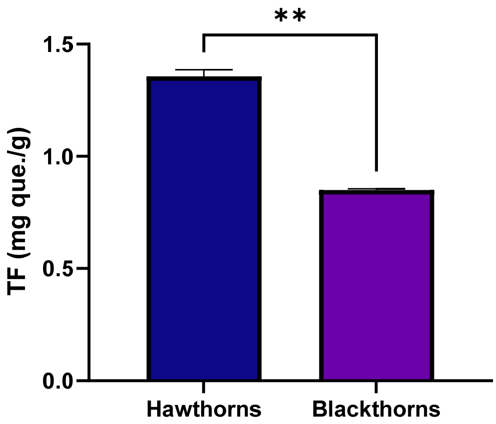
Blackthorn showed the highest content of vitamin E isomers and xanthophylls, with higher levels of lutein and zeaxanthin, compared with hawthorn.

The ratio between vitamin E isomers is also important regarding the antioxidant potential. Therefore, the ratio $(\gamma + \delta)/\alpha$ was calculated, and the results obtained were 0.26 for blackthorns and 0.25 for hawthorns. The obtained values were below 1, suggesting an enhanced antioxidant activity.

The content of the water-soluble antioxidants was also assessed, and the results are presented in Figure 2a, b. The data revealed that hawthorn exhibited the highest levels of the total polyphenols and total flavonoids, compared with blackthorn.



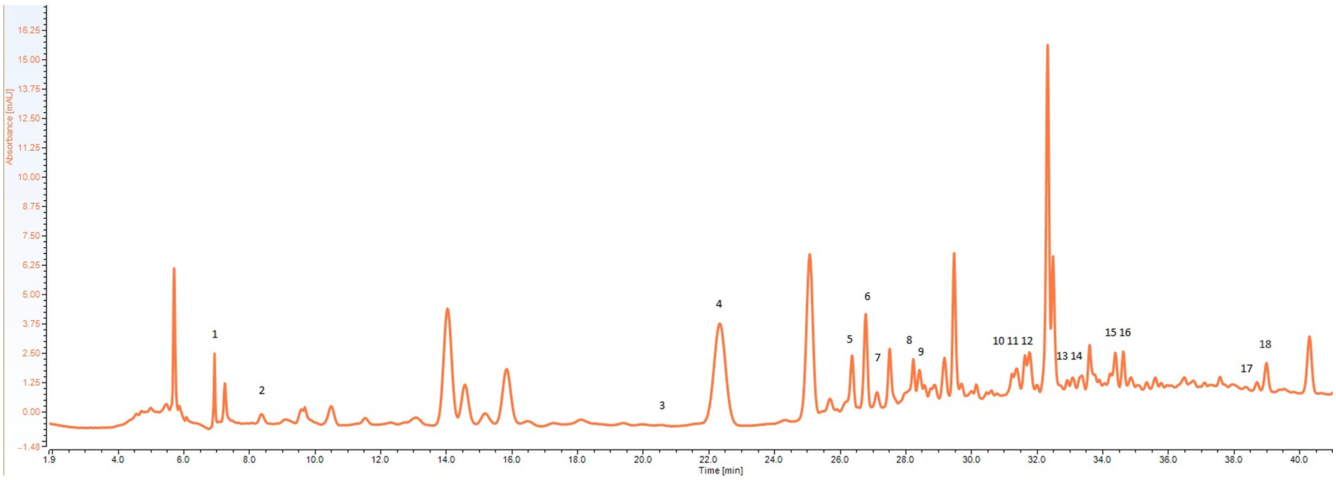
(**a**)



(**b**)

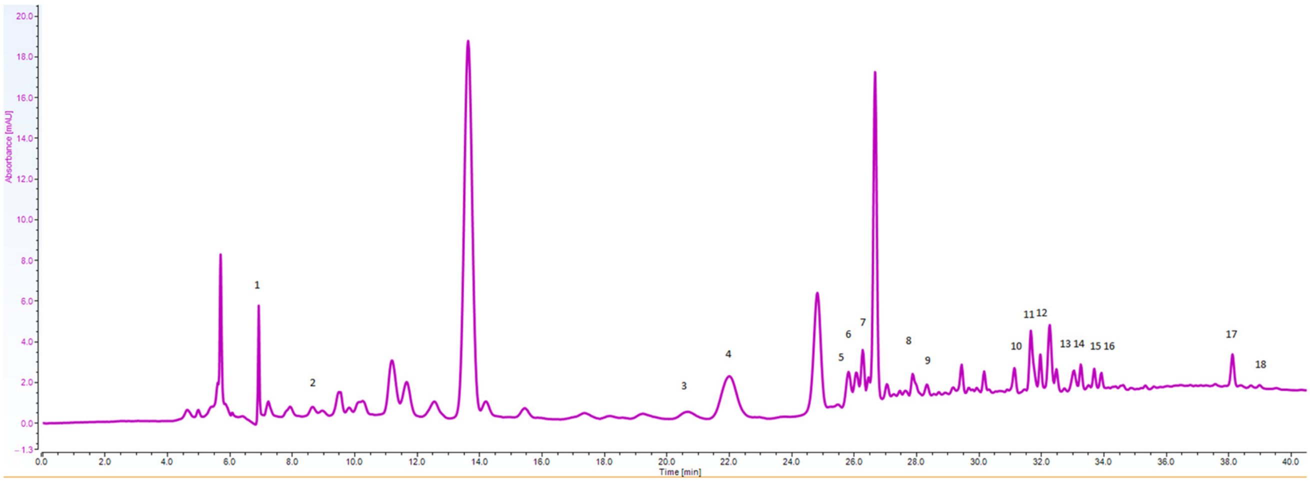
Figure 2. The total polyphenol content (**a**) and total flavonoid content (**b**) of blackthorn and hawthorn. The results are presented as means ± SEM; ** represents significant differences between means (** $p \leq 0.01$).

The individual profile of the polyphenols for hawthorns and blackthorns was also determined, and their representative chromatograms are presented in Figure 3a,b.



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Individual polyphenols profile for hawthorn (**a**) and blackthorn (**b**), where: 1—gallic acid, 2—epigallocatechin, 3—catechin, 4—chlorogenic acid, 5—vanillic acid, 6—caffeic acid, 7—syringic acid, 8—3-hydroxybenzoic acid, 9—epicatechin, 10—rutin, 11—p-coumaric acid, 12—ellagic acid, 13—4-hydroxy-3-methoxycinnamic acid, 14—ferulic acid, 15—protocatechuic acid, 16—resveratrol, 17—quercetin, 18—trans-cinnamic acid.

In our study, eighteen individual polyphenols were detected in hawthorn and blackthorn, divided in three different classes (Table 4). As it is shown in Table 4, hawthorns presented the highest content of the total polyphenols, with high concentration of chlorogenic and ellagic acids.

Table 4. Individual polyphenols profile of hawthorn and blackthorn (n = 3).

Polyphenols (mg/g)	Hawthorn	Blackthorn	SEM [1]	*p*
		Phenolic acids		
Gallic Acid	0.057 [a]	0.108 [b]	0.001	<0.0001
Vanillic acid	0.015 [a]	0.069 [b]	0.001	<0.0001
Caffeic acid	0.058 [a]	0.086 [b]	0.001	<0.0001
Syringic acid	0.051 [a]	0.066 [b]	0.001	<0.001
Hydroxybenzoic acid	0.173 [b]	0.054 [a]	0.002	<0.001
Protocatechuic acid	0.032 [b]	0.013 [a]	0.001	<0.001
Chlorogenic acid	0.324 [a]	0.459 [b]	0.007	<0.0001
Ferulic acid	0.005 [a]	0.017 [b]	0.001	<0.0001
Methoxy cinnamic acid	0.006 [a]	0.010 [b]	0.001	0.002
Trans Cinnamic acid	0.003 [a]	0.004 [b]	0.001	<0.0001
Ellagic acid	0.303 [b]	0.094 [a]	0.001	<0.001
Coumaric acid	0.018 [a]	0.031 [b]	0.017	<0.001
		Flavonoids		
Catechin	0.099 [a]	0.111 [b]	0.002	0.008
Epigallocatechin	0.234 [b]	0.196 [a]	0.005	0.006
Epicatechin	0.132 [b]	0.043 [a]	0.001	<0.0001
Rutin	0.057[a]	0.114 [b]	0.001	<0.0001
Quercetin	0.015 [a]	0.053 [b]	0.001	<0.0001
		Stilbenes		
Resveratrol	0.008 [b]	0.001 [a]	0.001	0.002
Total	1.59 [b]	1.53 [a]		0.001

[1] the standard error of the mean, [a,b] means within a row with no common superscript differs ($p < 0.05$).

In case of blackthorn, the results suggested that the major polyphenol presented in its composition was the chlorogenic acid. From the point of view of the studied polyphenols classes, hawthorns exhibited the highest concentrations of the total phenolic acids, total flavonoids, and stilbene, as it is shown in Figure 4.

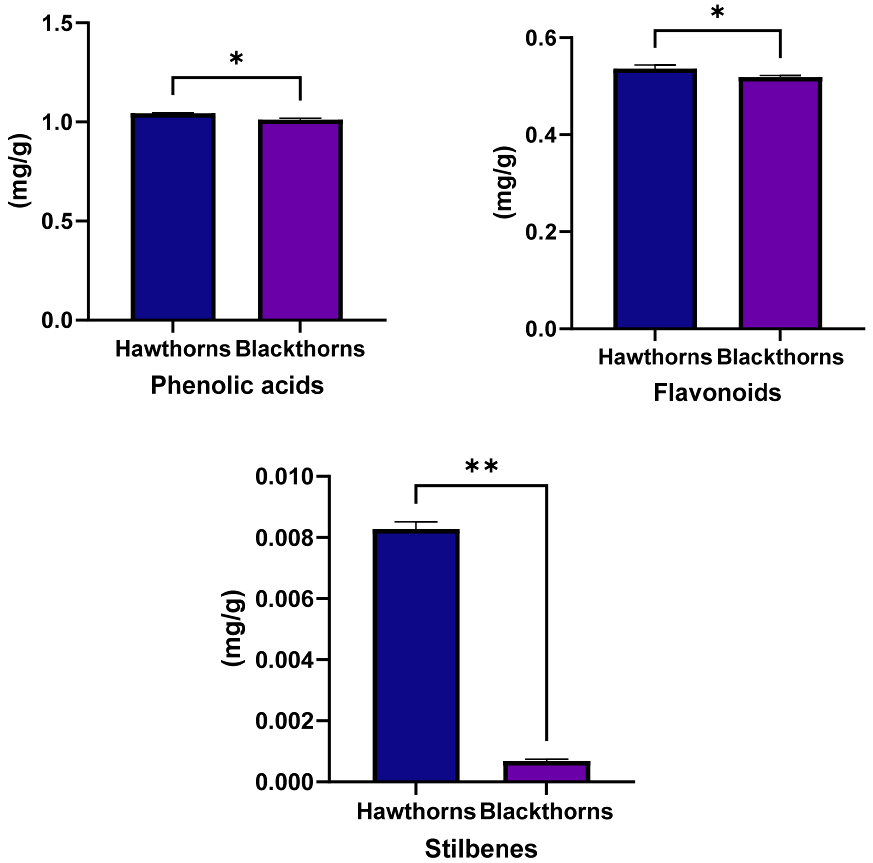


Figure 4. Polyphenols classes determined in hawthorns and blackthorns (phenolic acids, flavonoids, and stilbenes). The results are presented as means ± SEM; *, ** represents significant differences between means (* $p \leq 0.05$, ** $p \leq 0.01$).

3.4. Antioxidant Activity of the Studied Plants

The results describing the antioxidant activity of blackthorn and hawthorn, assessed through different three methods, are presented in Table 5.

Table 5. Antioxidant activity of blackthorn and hawthorn, assessed through three different methods (n = 3).

Parameters	Blackthorns	Hawthorns	SEM [4]	*p*
DPPH [1] (mM Eq. Trolox)	47.00 ^{b}	248.18 ^{a}	17.958	0.001
ABTS [2] (mM Trolox)	65.87 ^{b}	102.85 ^{a}	2.275	<0.0001
TAC [3] (mM Eq. Ascorbic acid)	49.56 ^{b}	97.61 ^{a}	4.014	0.001

[1] antioxidant capacity using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate method, [2] scavenging of samples against 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), [3] total antioxidant capacity using phosphomolybdenum method, [4] the standard error of the mean, ^{a,b} means within a row with no common superscript differs ($p < 0.05$).

The results suggested that hawthorn had the higher antioxidant potential, compared with blackthorn, with significantly higher levels of DPPH ($p = 0.001$), ABTS ($p < 0.0001$), and TAC ($p = 0.001$).

The free radical scavenging properties of blackthorn and hawthorns were determined against superoxide radicals (O_2^-), and the results are presented in Figure 5.

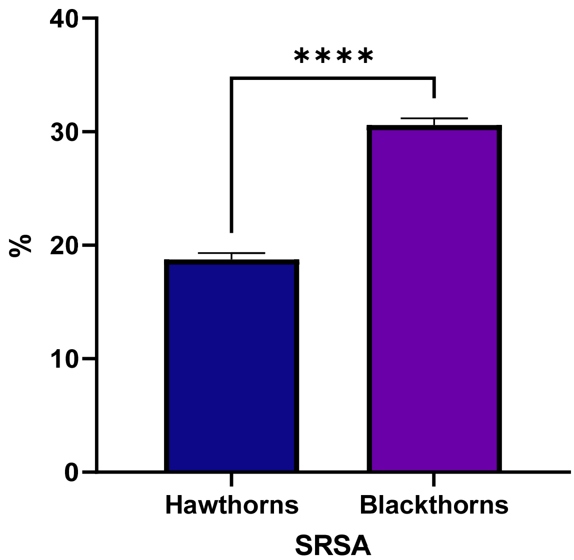
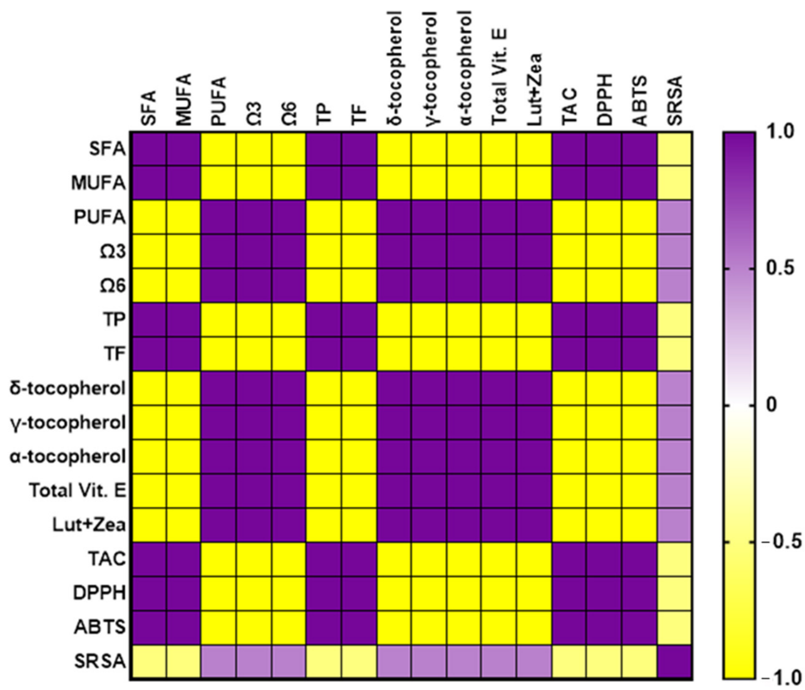


Figure 5. The scavenging capacity of blackthorn and hawthorn against superoxide radicals (SRSA). The results are presented as means ± SEM; **** represents significant differences between means (**** $p \leq 0.0001$).

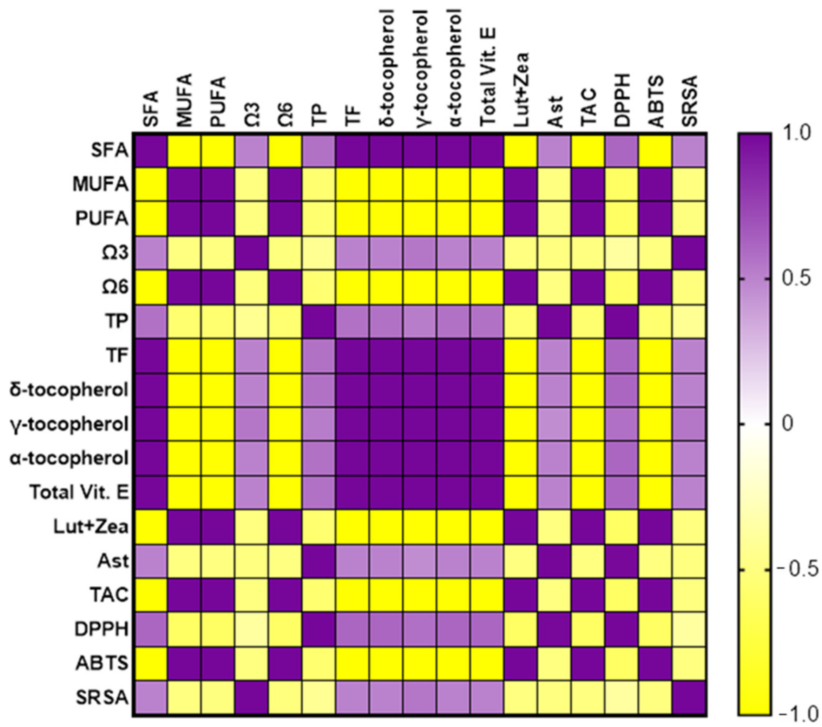
The results revealed that blackthorn exhibited the highest radical scavenging potential against superoxide radicals, compared to hawthorn (Figure 5). This fact indicated that blackthorn had a superior ability to neutralize superoxide radicals, highlighting its high antioxidant properties.

In Figure 6 is presented the Pearson correlation as heatmap between the fatty acids' composition (SFA, PUFA, MUFA, omega 6, and omega 3 fatty acids), antioxidant compounds (total polyphenols, total flavonoids, isomers of vitamin E, and xanthophyll), and antioxidant potential (total antioxidant capacity, DPPH, ABTS, and the scavenging potential against superoxide radicals) for the blackthorn (a) and hawthorn (b).



(**a**)

Figure 6. *Cont.*



(**b**)

Figure 6. Pearson correlation as heatmap between the fatty acids' composition, antioxidant compounds, and antioxidant potential for the blackthorn (**a**) and hawthorn (**b**). Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid, TPC, total polyphenols content; TF, total flavonoids; Lut+Zea, lutein and zeaxanthin, Ast, astaxanthin, TAC, total antioxidant capacity; SRSA, the scavenging against superoxide radicals.

The Pearson correlation for blackthorn (Figure 6a) indicated a strong positive correlation between PUFA, omega-3, and omega-6 fatty acids (r = 1.00). Moreover, PUFA content was positively correlated with all lipo-soluble antioxidants (vitamin isomers, lutein, and zeaxanthin; r = 1.00). The total content of polyphenols and flavonoids showed a highly positive correlation with TAC, DPPH, and ABTS (r = 1.00), while the lipo-soluble antioxidants were positively correlated with SRSA (r = 0.50–0.51). Negative correlations were observed between PUFA, omega-3, omega-6 fatty acids, and water-soluble antioxidants (total polyphenols and total flavonoids), as well as the parameters describing antioxidant activity (DPPH, ABTS, TAC), with r = −1.

For hawthorns (Figure 6b), the Pearson correlation revealed a strong positive correlation among the isomers of vitamin E (r = 1.00), as well as a significant positive correlation between omega-3 fatty acids and the isomers of vitamin E (r = 0.50–0.51). Additionally, the total polyphenols content was positively correlated with the total flavonoids content (r = 0.57). SRSA showed a positive correlation with both content of the total flavonoids and vitamin E isomers (r = 0.50). Also, negative correlations were observed for SRSA with MUFA and PUFA (r = −0.50).

4. Discussion

4.1. Proximate Composition and Trace Mineral Content

The analysis of the proximate chemical composition revealed that blackthorn has a higher content of crude protein and crude fat, whereas hawthorn has a higher content of crude fiber.

However, the crude protein level places them below feed ingredients scarce in proteins. The fat content offers less information on the energy value as this depends also on other factors, such as the content of non-structural ingredients, the digestibility of the organic matter, etc. At the moment, blackthorns and hawthorns are not typically used as feed ingredients, the ruminants' diets being formulated based on main nutrients content, such as protein. However, their high content of active substances makes them potentially valuable as feed additives for ruminants not only for productivity but also for improving the nutrient composition of products. The quality of meat and milk can be improved by nutritional strategies using natural feed additives (with a rich composition of antioxidant compounds) and their inclusion in the feed formula [27].

The total ash content did not present significant differences between the two plants. These findings are consistent with the data reported in the literature [28–31].

In the case of trace minerals, hawthorn showed a higher content of iron and zinc, while blackthorn had a higher content of manganese. The existing literature on the mineral composition of the studied plants indicated a wide range of variability for their mineral's concentration [9,32–34], which can be attributed to the plant species and the properties of the soil, as the latter significantly influences the trace-mineral composition of plants [35]. Trace-mineral content in ruminant diets is crucial and needs to be taken into account, considering that they are essential for many physiological processes that enhance animal health and productivity [36].

4.2. Fatty Acids Composition

The data regarding the fatty acids profile revealed the hawthorns' highest concentration of PUFA, as well as greater content of omega-3 and omega-6 fatty acids, compared with blackthorns. Although the fat content of both hawthorns and blackthorns are lower, compared with other sources of unsaturated fatty acids (UFA), the prevalence of oleic acid and cis-linoleic acid, respectively (more than 50% of the total FAME), raise a particular interest. These data are important, considering that several studies have demonstrated that populations with a diet rich in UFA have low rates of cardiovascular disease, diabetes, multiple sclerosis, and bronchial asthma [37]. Furthermore, from the recorded data, it was observed that hawthorns presented significantly higher concentration of cis-linoleic acid (CLA) and alpha-linolenic acid. The supplementation of ruminants with sources of essential fatty acids is crucial, considering that both CLA and α-linolenic acid can be precursors for the biosynthesis of dihomo-γ-linolenic acid, arachidonic acid (which is a precursor for the prostaglandins synthesis), and, respectively, for eicosapentaenoic acid (EPA) [38]. In terms of the MUFAs, oleic acid was the most abundant in both studied plants, with higher composition in blackthorns. This type of MUFA plays a crucial role in ruminant nutrition; as the literature suggested, increasing the concentration of oleic acid in milk can lead to decreases in levels of plasma cholesterol, LDL-cholesterol, and triacylglycerol concentrations. This, in turn, may reduce the risk of developing coronary artery disease for consumers [39]. Moreover, hawthorns presented higher content of medium-chain fatty acids C6-C12 (MCFA), being considered essentials for the energy metabolism and anabolic processes of the mammals [40].

4.3. Compounds with Antioxidant Activity

Both plants showed high levels of components with antioxidant activity, blackthorn having higher amounts of total vitamin E and its isomers compared to hawthorn. The predominant vitamin E isomer in both plants was alpha-tocopherol, with concentrations of 97.79 mg/kg in blackthorn and 51.77 mg/kg in hawthorn, known for its higher biological activity compared to gamma- and delta-tocopherol isomers [41]. The ratio between isomers is significant for antioxidant potential. Thus, the $(\gamma + \delta)/\alpha$ ratio was calculated, and the results obtained were 0.26 for blackthorn and 0.25 for hawthorn. In both plants, the tocopherol isomer ratios were below 1, indicating an enhanced antioxidant activity. This is consistent with the literature, which suggests that vitamin E isomers ratios near or below 1 indicate higher antioxidant activity, implying that a higher α-tocopherol content corresponds to an increased antioxidant activity [42]. Furthermore, blackthorn exhibited higher concentrations of xanthophyll, particularly lutein, whereas astaxanthin was only found in hawthorn. These compounds are important due to their antioxidant and anti-inflammatory properties with beneficial effects on human health and animals' nutrition [43].

In case of hydro-soluble antioxidants, hawthorn exhibited the highest levels of total polyphenols and total flavonoids. These findings contradict the results of [44], who found that blackthorn had the highest concentrations of these compounds compared to hawthorn. However, other studies have reported variable results regarding the polyphenol and flavonoid content in studied plants, showing a wide range of values. This variability can be mainly attributed to differences in plant species and geographic locations [45–47].

The results of the polyphenol profile revealed that hawthorns presented the highest composition of the individual polyphenols studied, compared with blackthorns. The most abundant polyphenol presented in its composition was the chlorogenic acid, which is consisted with the existing literature [47]. Also, in our study, it was observed that hawthorns presented high concentrations of ellagic acid. This type of polyphenol was also found in other fruit species, such as rose hip, and it possesses important practical applications, having antibacterial, anti-inflammatory, and antiviral effects [48]. In the case of blackthorn, the most abundant polyphenol presented in its composition was the chlorogenic acid, followed by epigallocatechin, rutin, and gallic acid, according to data presented in the literature [5]. The importance of the supplementation with polyphenols in ruminants' diet was highlighted in recent studies, as there is a high interest in enriching milk and meat with PUFA, which have beneficial effects on human health, but they are susceptible to oxidation [49]. Considering the economic and environmental arguments, there is a growing interest in using natural sources of polyphenols in animals' nutrition to prevent or to mitigate the oxidative process. Moreover, the inclusion of natural antioxidants in ruminants' feed can improve the quality of milk and meat and their nutritional value [50].

The assessment of the antioxidant capacity revealed that hawthorn exhibited the highest antioxidant potential based on DPPH, ABTS, and TAC results. This can be attributed to the higher concentrations of total polyphenols and flavonoids observed in hawthorn compared to blackthorn. However, these results contradict the data reported by [6], who observed a greater antioxidant potential in blackthorn. These disparities may also result from significant variability in antioxidant compositions within the same plant species and differences in geographic regions [6,47], which raise the need to analyze each batch for if the plants are to be valorized as feed ingredients/raw materials for developing feed additives. The assessment of the scavenging potential against superoxide radicals revealed that both plants possess remarkable inhibition power. However, blackthorn exhibited a significantly higher inhibition percentage, 61.26% greater than hawthorn. These results can be attributed to the higher content of lipo-soluble antioxidants found in blackthorn. These findings are consistent with literature, which highlights berries as potent-free radical inhibitors [51].

5. Conclusions

Our study showed that both studied plants presented high levels of bioactive compounds, with high relative concentrations of fatty acids that are crucial for human health and ruminants' nutrition. In terms of the antioxidant compounds, hawthorn was particularly noted as an important source of water-soluble antioxidant compounds, compared with blackthorn, especially in terms of the polyphenol content. On the other hand, blackthorn showed higher concentrations of water-soluble antioxidant compounds, specifically vitamin E, lutein, and zeaxanthin. Also, our study revealed that hawthorns exhibited the highest antioxidant potential, compared with blackthorn, based on DPPH, ABTS, and TAC results, while blackthorn presented a higher capacity for scavenging against the superoxide radicals. Our results suggested that hawthorn and blackthorn could be valorized in ruminants' nutrition as additives, considering their rich composition in bioactive compounds, with potential beneficial effects regarding the ruminants' nutrition and consumers' health.

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