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ORIGINAL RESEARCH PAPER

ANTIOXIDANT ACTIVITY AND PHYSICOCHEMICAL PARAMETERS OF YOGURT ENRICHED WITH EXTRACTS OF RED HAWTHORN (*CRATAEGUS MONOGYNA* JACQ.) AND BLACK HAWTHORN (*CRATAEGUS NIGRA* WALDST & KIT.)

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Abstract: The aim of this study was to determine some physicochemical parameters and antioxidant activity of fermented milks enriched with extracts of red hawthorn *(Crataegus monogyna* Jacq.) and black hawthorn (*Crataegus nigra* Waldst & Kit.) Antioxidant activity and the content of total phenols of water extracts of both species of hawthorn - red and black - were analyzed. The effect of the inclusion of the extracts in amounts of 5, 10 and 15 % of both species of hawthorn on the physicochemical and antioxidant properties of the obtained yogurts was monitored. Yogurts with the addition of red hawthorn extract (15 %) showed the highest phenolic content (TPC) and antioxidant properties determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) and FRAP (Ferric ion reducing antioxidant power) methods. Milk with extracts has better physicochemical properties (dry matter, syneresis and retention capacity). The use of hawthorn extracts in the production of yogurts is a new method for the development of dairy foods with functional properties.

Keywords: *antioxidant activity, hawthorn berries extracts, sensory analysis, total phenolics, yogurt*

INTRODUCTION

Hawthorn (C*rataegus* spp.) consists of approximately 1000 species of Rosaceae family and is cultivated in Europe, North Africa, Asia and North America [1].
Hawthorn species *Crataegus monogyna* Jacq. and *Crataegus nigra* Waldst & Kit. are widespread in Bulgaria. They are found at the edges of forests and along rivers.
Hawthorn berries are eaten fresh or processed into jams, jellies, soft drinks, candies and as preserved fruit. Hawthorn products are introduced as an alternative treatment for hypertension, angina pectoris and arrhythmia. Additionally, they can be used to treat indigestion, diarrhea, abdominal pain, etc. [2].
Berries contain valuable secondary metabolites, such as flavonoids, vitamin C, glycosides, anthocyanins, tannins, phenolic compounds, which have high levels of antioxidant activity [3, 4].
Crataegus berries are a source of phenolics in values up to 30.63 mg gallic acid equivalent (GAE)·g^{-1} in ripe berries. Kostić and Middleton reported that the content of total phenolics in ethanolic extracts of dried hawthorn fruits was 35.4 ± 2.48 mg $GAEg^{-1}$ dried mass [5, 6].
The use of medicinal plant raw materials and fruits in the food industry increases the nutritional and functional properties of food products enriching them with biologically active substances.
Yogurt is a fermented milk product with proven probiotic properties that is widely consumed in many countries. It is rich in bioactive peptides formed during fermentation, but it has a low antioxidant activity.
It has been proven that fermented milks enriched with plant extracts from grape and olive pomace have not only high antioxidant and antimicrobial properties, but also a longer shelf life [7, 8].
Many dairy producers use sodium or potassium sorbate to protect them from mould and yeast growth. According to Beltrán-Barrientos, artificial preservatives adversely affect human peripheral blood lymphocytes [9]. Therefore, replacing artificial preservatives with natural ones is imperative, using aqueous or ethanolic plant extracts with high antioxidant activity.
A number of researchers have investigated the influence of plant extracts added in the production of fermented milks, for example - artichoke extracts [10]; of *Lycium barbarum* [11]*;* of spirulina [12]; of black tea [13].
Following the new innovative trends in the dairy industry to create dairy products with functional properties, the objectives of the present investigation were to determine some physicochemical parameters and antioxidant activity of fermented milks enriched with extracts of red hawthorn (*Crataegus monogyna* Jacq.) and black hawthorn (*Crataegus nigra* Waldst & Kit.).

MATERIAL AND METHODS

Raw materials

In the present investigation, raw cow's milk was used, characterized by sensory, physicochemical and microbiological indicators according to the requirements of EU Regulation No. 853/2004. Fat content of the milk is standardized to 3.6 ± 0.1 %.
A yogurt starter containing the specific strains (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) purchased from company Laktina OOD, Bulgaria, was used.
Dried fruits of two species of hawthorn, red (*Crataegus monogyna*) and black (*C. nigra*) purchased commercially, were used. Prior to analyses, fruits were ground in a laboratory mill (Model PRO 02; 2600 rpm) and stored in double paper bags in a wooden cabinet at room temperature (20 ± 2 °C), away from direct sunlight or other heat sources. The fruit extracts are obtained by water extraction at hydro module 1:10, temperature 60 ºC and duration of the process 60 min. The extraction conditions were chosen based on literature data and preliminary unpublished studies of ours [14, 15].

Yoghurt preparation

The production of yogurt with the addition of hawthorn extracts is carried out according to the classic technology for the production of yogurt, namely: pasteurization of milk at 92 °C for 15 min, cooling to 42 °C, inoculation with 2 % sourdough, dosing in packages and adding the hawthorn extracts in amounts of 5, 10 and 15 % relative to the mass of milk and incubating at 42 °C until the samples reached *p*H 4.6. Ready samples of yogurt are stored at 4 ± 0.5 °C.
Yogurt samples were prepared for compositional analyses according to the Herrera method [16], by diluting 2.5 g of each sample in distilled water (10 mL); incubated at 45 ºC in a water bath for 10 min, then centrifuged to remove precipitated proteins. The resulting supernatants were stored at a temperature of – 20 ºC.
Yogurt samples for technological properties were not treated as described above but tested directly after their making.
The following fermented milk samples were obtained: $Y_{Control}$ – as control, $Y_{1\ red\ hawthorn}$ – 5 %, $Y_{2\ red\ hawthorn}$ – 10 %, $Y_{3\ red\ hawthorn}$ – 15 %, $Y_{4\ black\ hawthorn}$ – 5 %, $Y_{5\ black\ hawthorn}$ – 10 %, $Y_{6\ black\ hawthorn}$ – 15 %.

Physicochemical analyses of extracts of red hawthorn *(Crataegus monogyna)* and black hawthorn *(C. nigra)*

pH was measured potentiometrically with a *p*H-meter -7110 (Germany).

Tannins were determined by titration of the obtained extract with 0.1 N $KMnO_4$ under indigo carmine indicator, % [17].

Total phenolic compounds (TPC)

Total phenolic content was measured by a slight modification of the Folin-Ciocâlteu method. 1 mL of Folin-Ciocâlteu reagent (diluted five times) was mixed with 0.2 mL of the extracts and then 0.8 mL of 7.5 % Na_2CO_3 were added. After a reaction time of 20 min at room temperature (20 ± 2 °C) the absorbance of the solution was read at 765 nm with a UV/Vis Spectrophotometer (Perkin Elmer Lambda, 25, 101 NB, USA) against the blank. A standard curve of gallic acid solution (25, 100, 300, 400, 500, 600 and 700 $\mu g \cdot mL^{-1}$) was prepared using a similar procedure. The results were expressed as milligram equivalents of gallic acid (GAE) per mL extract [18].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was evaluated as the hawthorn extract (0.15 mL) was added to 2.85 mL of freshly prepared 0.1 mM DPPH solution in methanol. The samples were incubated for 15 min at 37 °C in darkness. The reduction of the absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol [13]. Radical scavenging activity of hawthorn extracts was expressed as mM Trolox equivalent (TE) per mL extract.

Ferric reducing antioxidant power assay (FRAP)

The FRAP method was performed as previously described [19]. 3 mL freshly prepared FRAP reagent (consisting of 10 parts 0.3 M acetate buffer (*p*H 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water) were mixed with 0.1 mL of the hawthorn extract. After 10 min at 37 °C in darkness, the absorbance was measured at 593 nm against blank prepared with the used solvent for extraction. The results were expressed as mM Trolox equivalent (TE) per mL extract.

Color of extracts

The color of plant extracts can sometimes change when they come into contact with certain solvents. This is because the solvent can react with the compounds present in the extract, forming new colored compounds. The choice of solvent used during the extraction process can therefore impact the final color of the extract.

A Galaxy A53 mobile phone video sensor (Samsung Electronics Co., Ltd., Seoul, South Korea) was used to obtain color digital images. The video sensor is 64 MP.

The resulting color digital images are in the RGB color model. They are converted to CIE L*a*b color model according to CIE Lab 1976 standard. Color component conversion functions at observer 2o and illumination D65 were used.

The color difference ΔE was determined. It varies in the range of 0 - 100, if closer it is to 0, the closer the colors of the extracts are to each other, and if closer it is to 100, the more they differ.

$$\Delta E = \sqrt{(L_c - L_a)^2 + (a_c - a_a)^2 + (b_c - b_a)^2} \quad (1)$$

where L_c, a_c, b_c are color components of the control sample; L_a, a_a, b_a are color components of the sample with additive.

All data were processed at a significant level of $\alpha = 0.05$.

Samples with and without additive of red (*Crataegus monogyna*) and black (*C. nigra*) hawthorn extracts)

Titratable acidity and pH of milk samples

Titratable acidity is determined by titration with 0.1N NaOH under the indicator phenolphthalein [20]. Active acidity is measured with a *p*H-meter.

Dry matter of yogurt samples was established according to BDS 1109-89 [21].

Syneresis (S) and water-holding capacity (WHC)

Syneresis was determined according to the modified method by Joung [22]. Briefly, 10 g of yogurt was weighed into 50 mL cuvettes and centrifuged at 4000 rpm for 10 min. After the centrifugation, an aliquot was divided; its mass was weighed and syneresis index was calculated according to Equation (2):

$$S = \frac{m_1}{m_2}.100, \% \quad (2)$$

where m_1 is the mass of the supernatant and m_2 is the mass of the yogurt sample, (g).

The water holding capacity (WHC) of the yogurt samples was determined according to the method proposed by Barkallah [12]. From each sample, 10 g of yogurt are centrifuged at 4000 rpm for 30 min (centrifuge ST 802A, China) WHC is calculated by formula 3:

$$W = \left(1 - \frac{W_1}{W_2}\right).100, \% \quad (3)$$

where W_1 is the weight of whey after centrifugation and W_2 is the weight of the yogurt, (g).

Total phenolic compounds

The determination of total phenolic compounds includes the use of Folin-Ciocâlteu reagents and sodium carbonate (Na_2CO_3). Total phenolics were determined using the modified Folin-Ciocâlteu procedure [23]. A mixture of 100 µL of supernatant, 100 µL of Folin-Ciocâlteu solution (1 mol·L^{-1}) was prepared and left for 5 min at room temperature, after which 300 µL of 10 % Na_2CO_3 is added. The resulting solution was kept at room temperature for 30 min. The absorbance was measured by a UV/Vis spectrophotometer. The results were expressed as mg GAE·mL^{-1}.

Determination of antioxidant activity

DPPH method – 250 µL of yogurt supernatant were stirred vigorously with 3 mL of DPPH and placed in the dark at room temperature for 30 min. A control sample was prepared in the same way, but instead of milk extract, 250 µL of distilled water was

added. The absorbance at 517 nm with a UV/Vis spectrophotometer is measured and the results are expressed as μmol TE·mL^{-1} [24].

FRAP analysis – was performed according to the method of Benzie and Strain with some modifications [19]. The resulting supernatant obtained by the method described above was mixed with 50 mL of acetate buffer, 5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 5 mL of $FeCl_3$. The resulting mixture is thermostated for 15 min at 37 ℃. It was then measured with a spectrophotometer at a wavelength reading of the colored product was read at 593 nm. The results are expressed in μmol TE·mL^{-1}.

Sensory evaluation

The sensory evaluation of the yoghurts was performed by calculating the points of a hedonic scale, as well as by a modified method introduced by Nakov [25]. The yoghurts were given estimates from 1 to 9 of their properties: aroma, taste, texture, aftertaste and appearance. The evaluators gave their estimates using a 9-point hedonic scale from 1 - "extremely unacceptable" to 9 - "extremely good". The sensory analyses were carried out on the first and 14th day after the preparation of the yoghurt.

53 respondents participated in the organoleptic evaluation of the product.

An organoleptic analysis was carried out to collect information about yogurt with an additive. The organoleptic analysis was conducted in such a way that there was complete anonymity and confidentiality for each respondent. All information, such as names, contact information, and all other forms of identification, was previously removed to obtain a formality whereby individual responses were obtained that were not identified to any respondent, hence confidentiality.

Respondent demographics were not recorded to protect respondent anonymity and confidentiality. The motive behind this was for all respondents to feel free and confident enough to give honest and unbiased answers.

Respondents were informed about the purpose of the study and were assured that their responses would remain confidential. The questionnaire was structured so that no personal identifiers were included in the information it collected.

The study was carried out according to Resolution № P2-0209/2022 of the ethics commission of FTT – Yambol, Trakia University Stara Zagora which complies with the directives for the ethics and research related to food testing approved by the EU and the participants have given their informed consent for participation [26].

Statistical analysis

Data from triplicate experiments were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (± SD). One-way ANOVA was applied in order to determine differences between samples and maximum estimation error at significance levels $p < 0.05$.

RESULTS AND DISCUSSION

The results of the analyses show that the different hawthorn species *Crataegus monogyna* and *C. nigra* show differences in terms of chemical characteristics (*p*H, tannin content, total phenolics and antioxidant activity) in hawthorn berries (Table 1).
The *p*H values in the obtained extracts were 4.60 for *Crataegus monogyna* and 4.52 for *C. nigra* and they are higher than those reported by Abolfaz (3.93 - *Crataegus monogyna* Jacq.; 3.17 - *Crataegus nigra*) [27]. The content of tannins in the obtained red and black hawthorn extracts are 0.70 % and 0.75 %, respectively, and these values are slightly higher than the tannin content of 50 % ethanol extracts [28].

Table 1. *The physicochemical content of extracts of red hawthorn (Crataegus monogyna* Jacq.*) and black hawthorn (Crataegus nigra* Waldst & Kit.*)*

Indicators	**Red hawthorn** (*Crataegus monogyna*)	**Black hawthorn** (*Crataegus nigra*)
*p*H	4.60 ± 0.15	4.52 ± 0.17
Tannins, [%]	0.70 ± 0.11	0.75 ± 0.09
TPC, [mg GAE·mL^{-1}]	24.2 ± 0.12	28.3 ± 0.08
Antioxidant capacity		
DPPH, [µmol TE·mL^{-1}]	358.2 ± 0.13	401.5± 0.10
FRAP, [µmol TE·mL^{-1}]	88.5 ± 0.10	122.2 ± 0.11

According to Svedström, tannins, phenolic compounds and flavonoids are a major group of bioactive compounds that are extracted from hawthorn berries [30].
The total phenolic content (Table 1) of black hawthorn extracts is slightly higher than that of red hawthorn (28.3 mg GAE·g^{-1} extract and 24.2 mg GAE·g^{-1} extract). This phenolic content in the berries of both hawthorn species is in accordance with the literature data [15].
Studies on the phenolic content of hawthorn fruits are variable and this is probably due to the different hawthorn species and climatic conditions of cultivation [30].
The antioxidant activity of aqueous extracts of hawthorn berries is due to the presence of phenolic compounds [31].
Methanolic and aqueous extracts of hawthorn berries have been shown to have DPPH radical scavenging and Fe^{2+} chelating activities. Higher Fe^{2+} chelating activity was observed in the methanolic extract than in the aqueous extract.
Black hawthorn extracts showed higher antioxidant activity as measured using the DPPH and FRAP methods. The obtained values for antioxidant activity are higher than those reported in the literature for ethanol extracts of hawthorn [32].
The aqueous extracts of *Crataegus monogyna* and *C. nigra* can be considered as sources of antioxidants with application in food products.
Figure 1 shows an overview of the obtained hawthorn extracts. The color of both extracts is visibly different. This difference is proven by measuring color components and by differences in the composition of both extracts.
Plants contain various pigments, such as chlorophyll, carotenoids, anthocyanins and flavonoids, which give them the characteristic colors of the extracts

a) Red hawthorn b) Black hawthorn

Figure 1. *Extracts of Hawthorn used in this study – general view*

Table 2 shows the color components of the extracts of the two species of hawthorn. A significant difference was observed in the L* and b* color components, while a* components of the extracts of the two hawthorn species were close in value.

Table 2. *Color components of red and black hawthorn extracts*

Hawthorn	**Red hawthorn**			**Black hawthorn**		
Color component	L*	a*	b*	L*	a*	b*
Value	32.86±0.28	32.86±0.28	32.86±0.28	32.86±0.28	22.12±0.73	39.4±0.7

The color difference is ΔE = 29.78, which shows that the color of the two extracts are clearly distinguishable and it is also due to the different composition of the two species of hawthorn, species, climatic and geographical conditions in which they grow.
The results of the physicochemical analysis of the milk samples obtained with the addition of hawthorn extracts are presented in Table 3.

Table 3. *Influence of extract content and storage time on the physical characteristics of sour milks*

Storage day	**Sample**	***p*H**	**Acidity [°T]**	**Syneresis [%]**	**WHC [%]**	**Dry matter [%]**
Day 1	$Y_{Control}$	4.52 ± 0.15	96 ± 0.15	60.80 ± 0.11	36.82 ± 0.18	12.56 ± 0.09
	$Y_{1\ red\ hawthorn}$	4.48 ± 0.12	98 ± 0.14	52.22 ± 0.13	33.54 ± 0.18	12.40 ± 0.17
	$Y_{2\ red\ hawthorn}$	4.43 ± 0.14	98 ± 0.16	56.62 ± 0.14	34.87 ± 0.17	12.40 ± 0.11
	$Y_{3\ red\ hawthorn}$	4.40 ± 0.16	100 ± 0.12	57.58 ± 0.14	42.58 ± 0.19	12.35 ± 0.12
	$Y_{4\ black\ hawthorn}$	4.46 ± 0.17	100 ± 0.10	52.58 ± 0.19	40.31 ± 0.14	12.36 ± 0.14
	$Y_{5\ black\ hawthorn}$	4.40 ± 0.11	102 ± 0.13	52.54 ± 0.11	41.52 ± 0.16	12.40 ± 0.13
	$Y_{6\ black\ hawthorn}$	4.32 ± 0.12	110 ± 0.11	53.84 ± 0.12	41.02 ± 0.14	12.96 ± 0.15
Day 14	$Y_{Control}$	4.25 ± 0.10	110 ± 0.14	51.45 ± 0.13	42.58 ± 0.11	12.65 ± 017
	$Y_{1\ red\ hawthorn}$	4.26 ± 0.09	112 ± 0.18	50.27 ± 0.15	38.45 ± 0.12	12.45 ± 0.12
	$Y_{2\ red\ hawthorn}$	4.22 ± 0.11	110 ± 0.15	50.85 ± 0.17	42.12 ± 0.14	12.56 ± 0.09
	$Y_{3\ red\ hawthorn}$	4.20 ± 0.17	102 ± 0.16	50.45 ± 0.11	44.50 ± 0.16	12.42 ± 0.11
	$Y_{4\ black\ hawthorn}$	4.28 ± 0.13	110 ± 0.17	50.28 ± 0.13	42.42 ± 0.17	12.45 ± 0.10
	$Y_{5\ black\ hawthorn}$	4.26 ± 0.14	118 ± 0.15	50.17 ± 0.16	44.52 ± 0.16	12.66 ± 0.17
	$Y_{6\ black\ hawthorn}$	4.18 ± 0.16	120 ± 0.16	50.08 ± 0.17	46.52 ± 0.17	13.42 ± 0.12

The addition of hawthorn berries extracts to yogurt did not significantly change these parameters. The active and titratable acidity of yogurts, dry matter content, syneresis and retention capacity were similar to the control, which is in accordance with the data found in the literature [14].

The acidity of the milk samples without and with the addition of hawthorn extract was monitored during storage. During the first day of storage, a slight decrease in the *p*H values was observed in the milk samples with the addition of hawthorn extracts compared to the control. The decrease was from 0.60 to 0.20 *p*H units in all samples. As the concentration of added extract increases, the decrease in *p*H also increases. The lowest *p*H (4.18) was observed in the milk samples with the addition of 15 % black hawthorn extract (Y6).

The phenolic components and oligosaccharides contained in the extracts may act as prebiotics and stimulate the growth of lactic acid bacteria and the breakdown of lactose into lactic acid.

At the end of the storage period (day 14), the decrease in *p*H of both the control and the samples with the addition of hawthorn extracts moved within close limits - from 4.25 in the control to 4.18 in the sample with the addition of 15 % hawthorn extract black hawthorn. This is probably due to subsequent acidification resulting from the residual activity of lactic acid bacteria in the milk samples.

The titratable acidity values for the storage period (1 - 14 days) also increased, and the highest values (120 ^{o}T) are observed at day 14 of storage in milk added with 15 % black hawthorn extract.

Syneresis is a common textural defect in fermented dairy products. It is an important parameter proving the quality of yogurts during storage. Syneresis is influenced by the ability of proteins to retain water in the casein structure of yogurt [14]. Consumers find syneresis an undesirable property of yogurt, and manufacturers are therefore looking for methods to prevent it. Some plant extracts have been shown to be good hydrocolloids because they contain a certain amount of soluble fiber [33].

The water-holding capacity (WHC) of yogurts represents the ability of fermented milks to retain whey.

Table 4 shows the results for the water-holding capacity of the milk at the beginning (day 1) and at the end (day 14) of cold storage. It is evident from the data that the syneresis is highest in the control sample in both measurement periods – 60.80 % on the first day and 51.45 % on the 14th day. When the percentage of added extract to milk increases, the syneresis increases on the first day of storage. The lowest values of syneresis (52.22 %) on the first day of storage were observed in milk with the addition of 5 % red hawthorn extract. At the end of the storage period, the syneresis values were the lowest in the sample with the addition of 15 % black hawthorn extract (50.08 %), and the highest in the control – 51.45 %. Hawthorn contains soluble fiber and pectin, therefore syneresis values are expected to be lower in milk with a higher percentage of extract added. Li prove that hawthorn is rich in pectin, which has a greater viscosity than that of other polysaccharides obtained, for example, from citrus or apple pomace [34].

The water-holding capacity of milk shows a reverse trend of syneresis. On the first day of storage, WHC was greatest in milk added with 15 % red hawthorn than in the control (42.58 % and 36.82 %). On the 14th day of storage, an increase in WHC values was

observed and they were the highest in milk with the addition of 15 % black hawthorn extract – 46.52 %. Similar results for low syneresis values were obtained by Herrera *et al.* 2018, when adding hawthorn and strawberry extracts to milk [16]. The syneresis reduction and WHC ability increase according to Elgammal *et al*., 2017, probably due to the protein network formed between the fiber and the casein [35].

The addition of various types of additives to milk, which are rich in fiber and hydrocolloids, increase the dry matter content and prevent syneresis. Table 4 shows that the highest percentage of dry matter is in sample Y_6 with the addition of 15 % hawthorn extract on the 14th day of storage - 13.42 %. In the remaining samples, the values of dry matter range between 12.42 to 12.66 %.

The content of total phenols and antioxidant activity (AO) in the resulting milks were monitored (Table 4). Hawthorn fruits and their extracts contain bioactive components. When the percentage of added hawthorn extract in milk increases, their phenolic content also increases. On the first day of storage, the highest phenolic content was found in the milk samples with the addition of 15 % black hawthorn extract (115.36 mg GAE·mL^{-1}). This content was 70 % higher than the control.

During the storage of the milk, the tendency to increase the total phenolic content in all samples was preserved, and this increase was within 3 - 4 %. Similar results were obtained by Zhang, when adding plant extracts to yogurts [36].

Total phenols improve antioxidant activity in foods; therefore, higher values of antioxidant activity are expected in milk as well.

High antioxidant activity according to both determination methods (DPPH and FRAP), higher values indicate black hawthorn milk. A slight increase in AO values was observed during milk storage, and in the samples with black hawthorn they reached 159.65 µmol TE·mL^{-1} by DPPH and 55.11 µmol TE·mL^{-1} by FRAP method, respectively.

Table 4. *Total phenolic content and antioxidant activity*

Storage day	Sample	Total polyphenols [mg GAE·mL^{-1}]	DPPH [µmol TE·mL^{-1}]	FRAP (µmol TE·mL^{-1})
Day 1	$Y_{Control}$	70.25 ± 0.09	92.51 ± 0.19	41.32 ± 0.19
	$Y_{1\ red\ hawthorn}$	82.45 ± 0.18	115.39 ± 0.14	45.64 ± 0.19
	$Y_{2\ red\ hawthorn}$	86.11 ± 0.16	120.32 ± 0.15	45.65 ± 0.15
	$Y_{3\ red\ hawthorn}$	89.36 ± 0.12	122.51 ± 0.16	46.89 ± 0.16
	$Y_{4\ black\ hawthorn}$	94.54 ± 0.13	130.23 ± 0.14	51.35 ± 0.17
	$Y_{5\ black\ hawthorn}$	95.34 ± 0.15	140.25 ± 0.17	52.69 ± 0.18
	$Y_{6\ black\ hawthorn}$	115.36 ± 0.19	148.39 ± 0.16	52.97 ± 0.15
Day 14	$Y_{Control}$	71.28 ± 0.11	120.34 ± 0.13	45.39 ± 0.14
	$Y_{1\ red\ hawthorn}$	86.54 ± 0.15	123.56 ± 0.15	46.25 ± 0.18
	$Y_{2\ red\ hawthorn}$	89.14 ± 0.13	130.57 ± 0.17	47.29 ± 0.14
	$Y_{3\ red\ hawthorn}$	93.56 ± 0.12	135.95 ± 0.18	52.33 ± 0.16
	$Y_{4\ black\ hawthorn}$	92.49 ± 0.12	156.82 ± 0.16	53.98 ± 0.17
	$Y_{5\ black\ hawthorn}$	97.36 ± 0.11	158.61 ± 0.13	54.87 ± 0.19
	$Y_{6\ black\ hawthorn}$	120.69 ± 0.14	159.65 ± 0.17	55.11 ± 0.17

Good stability of antioxidant activity in yogurt with extracts from plant raw materials was also observed by other authors. Dabija *et al*., 2018 and Zhang *et al*. 2019, proving the antioxidant stability of yogurts enriched with herbal extracts, they noticed that it

increased during storage. Raikos, also demonstrated that lactic acid drinks enriched with salal fruit and blackcurrant pomace extracts retained their antioxidant capacity during storage [37].

During milk storage, bioactive peptides are formed as a result of proteolysis, which leads to increased activity of lactic acid bacteria, which can increase antioxidant activity [38].

Antioxidant activity was preserved during storage and even increased in some samples. It can be appreciated that lactic acid and herbal extracts affect the stability of the product over time. During yogurt storage, there is a continuous release of phenolic compounds from the yogurt matrix due to the breakdown of cell walls and membranes of the starting materials (e.g., milk, fruits, and berries). These phenolic compounds are known for their antioxidant properties and contribute to the overall antioxidant capacity of the yogurt. Some yogurt bacteria, such as Lactobacillus and Streptococcus species, produce antioxidant compounds like vitamins C and E, organic acids, and other bioactive molecules during fermentation. As yogurt ages, the metabolic activities of these bacteria may continue to produce these antioxidants, contributing to the observed increases in TP, DPPH, and FRAP.

During the storage of the milk, the tendency to increase the total phenolic content in all samples was preserved, and this increase was within 3 - 4 %. Similar results were obtained by Zhang *et al*., 2019, when adding plant extracts to yogurts [36].

Total phenols improve antioxidant activity in foods; therefore, higher values of antioxidant activity are expected in milk as well.

High antioxidant activity according to both determination methods (DPPH and FRAP), higher values indicate black hawthorn milk. A slight increase in AO values was observed during milk storage, and in the samples with black hawthorn they reached 159.65 µmol TE·mL^{-1} by DPPH and 55.11 µmol TE·mL^{-1} by FRAP method, respectively.

Good stability of antioxidant activity in yogurt with extracts from plant raw materials was also observed by other authors. Dabija and Zhang (2018), proving the antioxidant stability of yogurts enriched with herbal extracts, they noticed that it increased during storage [14, 36]. Raikos *et al*., 2019 also demonstrated that lactic acid drinks enriched with salal fruit and blackcurrant pomace extracts retained their antioxidant capacity during storage [37].

During milk storage, bioactive peptides are formed as a result of proteolysis, which leads to increased activity of lactic acid bacteria (streptococci), which can increase antioxidant activity [38].

Sensory analysis of the resulting milks was performed and the results are presented in Figures 2 and 3. The results of the sensory evaluation include aroma, taste, texture, aftertaste and appearance on days 1 and 14 of storage. On day 1 of the analysis, the panelists rated the milk samples, giving a higher rating to the control and the milk added with 15 % black hawthorn extract.

The addition of hawthorn extracts did not significantly affect the color, taste and aroma of the milk.

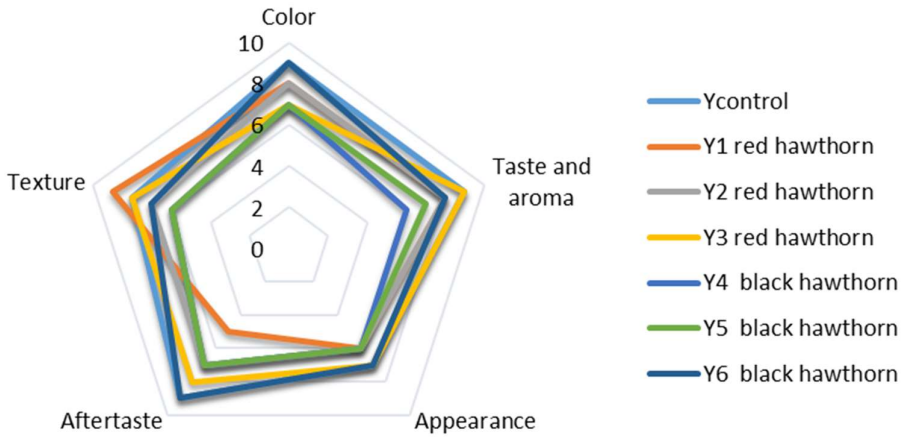

Figure 2. *Sensory evaluation of samples on day 1 of storage*

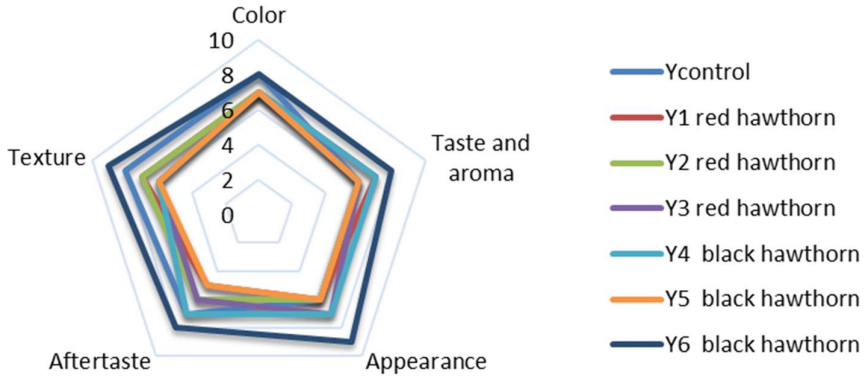

Figure 3. *Sensory evaluation of samples on day 14 of storage*

The overall result of the sensory evaluation of the milk on day 1 of storage was high for all samples.

In the last 14th day of storage, in the sensory evaluation of the milk with the best indicators of taste, aroma and texture, there is the sample with the addition of black hawthorn extract, and the control sample had the lowest results. Diacetyl and acetaldehyde, which give the typical taste of fermented milk products, decrease during the storage period, therefore the typical yogurt taste is also absent during the control [39].

CONCLUSION

The addition of hawthorn extracts into fermented milks is an effective method to improve their functional and nutritional properties.

The addition of hawthorn extracts to milk reduces syneresis, and the retention capacity increases compared to the control sample.
The addition of black hawthorn extracts in the amount of 15 % increase the concentration of total phenols and antioxidant activity of milk, therefore it can be considered as an antioxidant additive.
According to the data from the sensory evaluation of the milk, the milk with black hawthorn extracts has the best indicators related to texture and taste.
Black and red hawthorn extracts can be considered as an effective ingredient for the functional reinforcement of foods and for obtaining value-added products.

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