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Influence of the addition from aromatic plants on the quality of milk by Bulgarian Rhodopes cattle

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Abstract

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The purpose of the study was to determine the influence of biologically active additives in different concentrations on the organoleptic evaluation, physicochemical and fatty acid composition, antioxidant activity and total polyphenols of cow's milk for drinking, obtained from highly productive cows by Bulgarian Rhodope breed. Adding aromatic plant additives in different concentrations, improves the qualitative and quantitative composition of milk. The organoleptic evaluation of the studied milk gives us a clear idea, that the application of a higher concentration of additives worsens its smell, taste, colour, aroma and texture, but enriches it with fibbers, minerals and fats, as a result of which the energy value also increases. Drinking milk with the addition of aromatic plants in dry form has a reduced content of saturated fatty acids, and was enriched with biologically active fatty acids, which in turn leads to health benefits from their consumption, and was expressed by lowering AI and TI and increasing cholesterolemic index. The antioxidant activity of milk was improved and the total content of polyphenols was increased, with the highest values, obtained when adding turmeric with black pepper.

Keywords: fibber; antioxidant activity; polyphenols; indices

Abbreviations: B – basil; M – lemon balm /*Melissa officinalis*/; P – peppermint; T – thyme; R – rosemary; C – turmeric*:* black pepper; FAME – fatty acids methyl ester; F – fat; D-density; C – conduction; S – soluble non-fat (SNF); P – protein; W – water addition; L – lactose; FP – freezing temperature (point); S – salt (ash); pH – active acidity; E – energy; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; MCT – middle chain fatty acid; SCT – short chain fatty acid; BFA – branched fatty acid; FA – fatty acid; LPS – lipid preventive scor; AI – aterogenic index; TI – trombogenic index; h/H – hyper-hypocholesterolemic index; TFA – trans fatty acids; DPPH – 1,1-diphenyl-2-picrylhydrazyl; TPC – total phenolic content

Introduction

Milk is an important component of functional foods, as it satisfies the needs of the human body for the main nutritional and biologically active substances – complete proteins, lipids, a wide variety of mineral substances (Ca, Mg, Fe, Zn), and vitamins of group B – B6, B12; D, thiamin, riboflavin, etc., essential amino acids and fatty acids – CLA, ω–3 and ω–6 fatty acids, enzymes, etc. Nikolov et al. (2011) found in their studies of collective milk, obtained from the breeds of Bulgarian black spotted cattle and Bulgarian Rhodope cattle, during extensive rearing and feeding with winter ration, that there were no significant differences in relation to the content of solid non-fat – free residue, soluble protein, temperature freezing, pH and calcium content. The milk obtained from the cows of the Bulgarian Rhodope cattle breed has a significantly higher density (2.4%, $P < 0.05$), fat content (7.9%, $P < 0.05$), total protein (4.7%, $P < 0.05$), higher juice treatable acidity (15.1%, $P < 0.001$), higher curability, and syneresis in the production of white brine cheese. From a technological point of view, it has significantly more favourable quality indicators for the production of biologically clean and naturally rich cheeses. Ivanova et al. (2020), in a study of milk from cows of the Bulgarian Rhodope cattle breed, found a fat content from 3.24 to 5.10%, and relatively constant protein and lactose content. Total solids increased during lactation from 12.16 to 13.39%. The solid non-fat decreased in the middle of the considered period to 4.71, and increased to 8.31% at the end of lactation. Wangdi et al. (2016) were studied cow milk parameters in different seasons from different breeds, and obtained the following results: solid non-fat from 8.35 to 8.61%, fat from 4.80 to 5.11%, protein from 3.05 to 3.14%, freezing point (-0.52) to (-0.55)°C.

Fortification of milk with herbs would allow to obtain functional dairy products of high quality and increase their consumption (Oraon et al., 2017). Basil is an aromatic plant with anticancer, radioprotective, antimicrobial, anti-inflammatory, immunomodulatory, antistress, antidiabetic, antipyretic, antiarthritic and antioxidant effects (Shahrajabian et al., 2020). Lemon balm has antimicrobial activity (antiparasitic, antibacterial, antiviral, etc.), antispasmodic and insomnia properties, antioxidant activity determined by the content of flavonoids, rosmarinic acid, gallic acid, phenolic content, which prevent and treat diseases related to oxidative stress (Miraj et al., 2017). Peppermint was also used in the treatment of the upper respiratory tract in bronchitis. It acts as an antiseptic and pain reliever (Akbari et al., 2015; Baker et al., 2018; Khursheed et al., 2017; McKay and Blumberg, 2006; Meamarbashi and Rajabi, 2013). In folk medicine, peppermint is a suitable herb for the treatment of dizziness, insomnia, headaches, depression, epilepsy, colds, coughs, sinus infections, fatigue, shock, nervous tension, skin rashes, bruises, boils and menstrual disorders. It is widely used in the food and confectionery industry, for the production of soft drinks, in cosmetics for perfumes, soaps, toothpastes and mouthwashes and aroma therapy. Thyme has been used since ancient times for its culinary, aromatic and medicinal properties (Aljabeili et al., 2018). Rosemary is an aromatic plant with applications in the food, pharmaceutical and cosmetic industries, which has antitumor, antioxidant and antimicrobial activity (Oliva et al., 2022). Turmeric is a perennial herbaceous plant of the ginger family native to South India and Indonesia, known as "Indian saffron". It is widely used in the food industry, pharmaceutical and cosmetic industries. It has characterized by anti-inflammatory properties, relieves the symptoms of arthritis and intestinal problems. It has used medicinally for long-term and has taken internally as a stimulant. The combination of turmeric and black pepper in warm milk was a good source for sore throats, coughs, colds and other acute respiratory infections. Turmeric contains the lipophilic bioactive compound curcumin with antioxidant and anti-inflammatory properties (Hewlings and Kalman, 2017).

The use of aromatic plants as a dry additive in drinking milk was not well studied, and the literature data has mainly related to the addition of oils from them, but they were also scarce. Therefore, their in-depth study has necessary.

The purpose of the study is to determine the influence of biologically active additives in different concentrations on the organoleptic evaluation, physicochemical and fatty acid composition, antioxidant activity and total polyphenols of cow's milk for drinking, obtained from highly productive cows by Bulgarian Rhodope breed.

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Material and Methods

Collected milk from highly productive Bulgarian Rhodope cattle cows was used, taken every month for one year from the Research Centre of Stockbreeding and Agriculture, Smolyan. The milk was pasteurized, and 1 l of milk was used to add additives from aromatic plants in the form by dry substance in a concentration 0% – control (K) and 0.05%, 0.1%, 0.2% and 0.3%. The different types of additives and their concentration when added to milk were presented in Table 1.

The technological scheme is presented in Figure 1.

An organoleptic evaluation of the milk without and with an additive in different concentrations was performed to determine the most suitable concentration, based on the sensory perceptions of consumers (10 units). The physicochemical

Table 1. Addition of aromatic plants to drinking milk

Supplement	0.05%	0.1%	0.2%	0.3%
Basil	B1	B2	B3	B4
Lemon balm	M1	M2	M3	–
Peppermint	P1	P2	P3	–
Thyme	T1	T2	T3	–
Rosemary	R1	R2	R3	–
Turmeric:black pepper 3:1	C1	C2	C3	C4

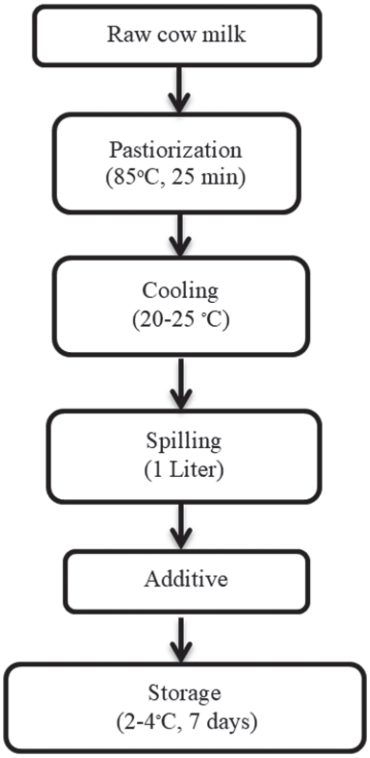

Fig. 1. Technological scheme for obtaining milk with additives

and fatty acid composition of the control and treated milk was performed at 24 h. Physicochemical analysis was performed with Lactoscan SP. Milkotronik Ltd. fibbers were analysed using an automatic fibber extraction system Dosi-Fiber-Selecta, Spain.

Extraction of total lipids was performed according to the method of Roese and Gottlieb. Fatty acid methyl esters (FAME), were analysed using a Shimadzu-2010 gas chromatograph (Kioto, Japan), equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on a CP 7420 capillary column (100 m × 0.25 mm i.d. 0.2 µm film, Varian Inc., Palo Alto. CA). Hydrogen was used as the carrier gas, and as a make-up gas – nitrogen. Four-step furnace mode was programmed – the column's initial temperature was 80°C/min, maintained for 15 min, then increased by 12°C/min to 170°C and maintained for 20 minutes, followed by a further increase of 4°C/min 186°C for 19 min and up to 220°C with 4°C/min, until the process is complete. The qualitative assessment of the fat fraction of the resulting samples includes the following: lipid preventive score (LPS), atherogenic (AI) and thrombogenic index (TI) (Ulbricht and Southgate, 1991), the ratio between hyper- and hypocholesterolemic (h/H) fatty acids, trans fatty acids (TFA) and the amount of saturated fatty acids (Regulation (EC) No 1924/2006).

$$LPS = FAT + 2 \times SFA - MUFA - 0.5PUFA$$
$$AI = 12:0 + 4 \times 14:0 + 16:0/[\Sigma MUFAs + PUFAn\text{-}6 + PUFAn\text{-}3]$$
$$TI = (14{:}0 + 16:0 + 18{:}0)/[0.5 \times \Sigma MUFAs + 0.5 \times PUFAn\text{-}6 + 3 \times PUFAn\text{-}3 + PUFAn\text{-}3/PUFA\ n\text{-}6]$$
$$h/H = (C18:1n\text{-}9 + C18:1n\text{-}7 + C18:2n\text{-}6 + C18:3n\text{-}3 + C18:3n\text{-}6 + C20:3n\text{-}6 + C20:4n\text{-}6 + C20:5n\text{-}3 + C22:4n\text{-}6 + C22:5n\text{-}3 + C22:6n\text{-}3)/(C14:0 + C16:0)$$

Preparation of samples for analysis of total phenol content and antioxidant activity

Milk without and with additives was extracted with 95% ethanol at a ratio of sample: extractant – 1:5 (w/v) for 6 h, at room temperature and in the dark. All samples after centrifugation (10°C. 4000 rmp. 10 min) and filtration (Whatman No. 4 paper) were stored at -20°C for subsequent analyses.

Determination of antioxidant activity

The antioxidant capacity of milk was evaluated by determining 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, according to the method of Brand-Williams et al. (1995), with a slight modification: 0,6 mL of a 0.2 mM solution of DPPH in methanol was mixed with 0.9 mL of methanol, and 0.5 mL of the corresponding sample dilution. Absorbance was measured after standing (60 min) at room temperature in the dark with a UV-Vis spectrophotometer (Biochrom Libra S20, UK), at 517 nm against methanol. In the control, the sample solution was replaced by 0.5 mL of 80% methanol. The antioxidant activity was calculated against the Trolox standard curve, and the results were expressed as milligrams Trolox equivalents per 100 g of product – mg TE/100 g of product.

Determination of the content of total phenols in milk

For the quantitative determination of the total phenolic content (TPC), the method of Singleton et al. (1999) was used with modification by Valyova et al. (2012). Briefly, 3.0 ml of distilled water and 0.25 ml of Folin–Ciocalteu reagent were added to 0.5 ml of the sample (with the corresponding dilution). After standing for 2 min, 0.75 ml of 20% sodium carbonate solution, and 0.5 ml of distilled water were added to the mixture. Absorbance was measured at 765 nm (on a UV-Vis spectrophotometer, Biochrom Libra S20, UK) after standing in the dark at room temperature for 120 min. TPC was calculated according to the standard gallic acid law, and was expressed as milligram equivalents of gallic acid per 100 g of product (mg GAE/100 g product).

Statistical analysis

Research results were analysed using the statistical program MiniTab 17, ANOVA and Tukey's post hoc test. Data were presented as mean and standard deviation (SD). A significance level of $p < 0.05$ was accepted for all comparisons.

Results and Discussion

The organoleptic research carried out by consumers, gives us information about the change of five indicators when using different types of aromatic plant additives. Basil, as an aromatic plant added to drinking milk was evaluated with the best organoleptic indicators at 0.5%, well acceptable at a concentration of 0.1 and 0.2%, and was evaluated less well when using 0.3%, Lemon balm, as an additive affects the evaluator perceptions, and they rated the milk with 0.05% additive as the most acceptable compared to the other variants. The addition of peppermint to milk gives the best assessment of smell, taste, aroma and colour at 0.05% concentration, consistency at 0.1% and the least evaluated parameters at 0.3%. The use of thyme in milk also resulted in the most acceptable organoleptic parameters for the senses at 0.1% addition and the least rated at 0.3%. The addition of rosemary to drinking milk had the most sensory-acceptable performance at 0.5%, followed by 0.1% and 0.2% supplementation. The combination of turmeric and black pepper added to milk has the most acceptable organoleptic indicators, when adding 0.05 and 0.1% of the additive, while at 0.2 and 0.3% the organoleptic indicators start to deteriorate, but they are still acceptable to consumers (Table 2).

Table 2

Idowu-Adebayo et al. (2021a) were investigated the effect of turmeric supplementation in soymilk, and hibiscus beverage on organoleptic parameters and consumer acceptability. Idowu-Adebayo et al. (2021b) were established the nutritional value and antioxidant activity of soymilk and a hibiscus-based beverage with turmeric supplementation. Idowu-Adebayo et al. (2022) were investigated the effect of the addition of turmeric paste to soy milk with and without heat treatment, increasing the nutritional and chemical value of all variants, and reported an increase in protein, iron, zinc, TPC (total phenol content) and antioxidant activity. Physical properties of turmeric like bulk density, true density, porosity and coefficient of static friction were determined by Athmaselvi and Varadharaj (2002). The geometric properties of turmeric were studied by Balasubramanianet al. (2022).

Table 2. Organoleptic evaluation of drinking milk with additives, n = 10

	K		B1		B2		B3		B4		M1		M2		M3		P1		P2		P3	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Smell	3.90^{G*}	0.99	3.80	0.79	3.40	0.70	3.70	1.16	3.10	0.74	3.60	0.84	3.30	0.95	2.80	0.92	3.80	1.03	3.50	1.08	3.00	0.94
Taste	4.10^{GIJPST}	0.99	3.90	0.74	3.60^{BD}	0.97	3.30	0.95	2.50	0.85	4.00^{EG}	0.67	2.50	1.08	2.80	1.03	3.60	1.07	2.70	1.16	2.80	1.23
Aroma	3.90^{CFGJPT}	0.88	3.90^{AC}	0.74	3.50^{BD}	1.08	3.10	0.74	2.50	0.85	3.50^{EG}	0.97	2.90	1.10	2.60	0.97	3.60	1.07	3.40	1.07	2.80	1.03
Consistency	4.20	1.03	4.20	0.79	4.00	1.05	4.10	0.99	3.50	0.97	4.20^{EG}	0.63	4.10	0.99	3.40	0.97	4.00	0.94	4.20	1.03	3.50	0.97
Color	4.30	1.06	4.30	0.82	4.30	0.95	3.70	1.42	2.80	0.92	4.00	0.82	3.50	1.43	3.40	0.84	4.10	0.99	4.00	1.25	3.50	0.85

1 – unacceptable; 2 – acceptable; 3 – I like it; 4 – I really like it; 5 – I like it very much; * Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance

Table 2. Organoleptic evaluation of drinking milk with additives, n = 10 (continue)

	T1		T2		T3		R1		R2		R3		C1		C2		C3		C4	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Smell	3.60	0.97	3.80	1.03	3.40	1.07	3.90	0.99	3.60	1.17	3.20	1.14	4.20	1.03	3.70	1.06	3.50	1.18	3.20	1.14
Taste	4.00	0.94	4.10	0.99	3.60	1.07	4.10^{NP}	0.99	3.70	1.16	3.00	1.25	4.20^{QST}	1.03	3.90^{RT}	0.88	2.90	1.29	2.70	1.25
Aroma	3.80	1.03	3.90	0.99	3.20	1.14	4.00^{NP}	0.94	3.40	1.17	3.00	1.05	3.90^{QT}	0.88	3.60	0.97	3.20	1.14	2.80	1.03
Consistency	4.10	0.99	4.30	1.06	3.80	0.92	4.40	1.07	4.00	1.05	3.70	1.06	4.30	1.06	4.10	0.99	3.70	1.06	3.40	1.07
Color	4.20	1.03	4.30	1.06	3.70	0.95	4.40	1.07	3.90	1.10	3.70	0.95	4.40	1.07	4.10	0.99	3.80	0.92	3.60	0.84

1 – unacceptable; 2 – acceptable; 3 – I like it; 4 – I really like it; 5 – I like it very much; * Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance

The total fat in the examined bulk tank cow's milk at 24 h averaged 3.24% in the control group, and increased with the addition of aromatic plants. The use of basil, lemon balm and oregano leads to an increase in the fat content by milk, while with peppermint it increases at 0.5% and has maintained at 0.1 and 0.2% supplementation, with thyme a constant fat content was found, regardless of additive concentration, an increase in fat content was found in turmeric at 0.5 and 0.3% additive, while at 0.1 and 0.2% it did not change compared to control milk. Additions, resulted in a decrease in milk density except for the addition of 0.1 and 0.2% mint, and 0.05, 0.1 and 0.2% turmeric (Table 3).

The conductance in the studied pooled cow's milk with the addition of peppermint in different concentrations increased significantly ($p < 0.05$). The highest value for it was found at 0.1% basil – 5.06 mS/cm, 0.05% lemon balm – 5.01 mS/cm, 0.2% peppermint – 5.17 mS/cm, 0. 1% thyme – 4.97 mS/cm ($p < 0.05$), 0.2% rosemary – 5.40 mS/cm and 0.2% turmeric – 4.89 mS/cm. The solid non-fat residue increased with the addition of peppermint at all concentrations, compared to the control milk. Application of 0.05% basil slightly increased SNF and at 0.1; 0.2 and 0.3% decreases. The addition of lemon balm and thyme ($p < 0.05$) the SNF content remains relatively constant regardless of the concentration of the supplement, but lower than the control milk group. The use of rosemary leads to a decrease in SNF with increasing a concentration compared to control milk. Turmeric supplementation resulted in significantly ($p<0.05$), higher SNF content at 0.05, 0.1 and 0.2% versus 0.3% supplementation. The protein content after addition was maintained at 0.05% addition and decreases with increasing concentration compared to control milk, in case of lemon balm, regardless of the applied concentration, the protein was maintained as a value, but was lower compared to control milk. The presence of water addition in the control and enriched milk with additives was not considered. Lactose decreased when adding 0.2 and 0.3% basil, at all concentrations of lemon balm and thyme, at 0.5% peppermint, but at other concentrations, it increased slightly compared to control milk. At 0.05% rosemary lactose was maintained and decreased at the higher concentrations and with turmeric, the lactose content was higher at 0.05, 0.1 and 0.2% additions, and remained at 0.3% compared to the control milk. Changes in the fat, protein and lactose content of supplemented milk were caused by the protein and fat content of the supplement itself and the carbohydrates in it, as well as the interaction between the individual type of supplement and the milk. The use of basil, lemon balm, thyme and rosemary resulted in an increase in the freezing temperature of the milk, while peppermint and the combination of turmeric and black pepper kept it close to the freezing temperature of the control milk. The mineral content of supplemented milk was maintained with the addition of basil except for 0.3% supplement – 0.94%, peppermint, rosemary and the combination of turmeric and black pepper, while with lemon balm and thyme it decreased compared to the control milk. Significant changes were found in the peppermint and turmeric group ($p < 0.05$). The active acidity of milk with additives was relatively constant depending on their concentration and type compared to the control group of milk, with the exception of 0.3% addition from basil – 6.75 and 0.2% rosemary – 5.68. The introduction of aromatic plants in the form of a dry substance leads to the enrichment of milk with fibber. Milk has the highest fibber content at 0.2% rosemary – 8.49%. The energy value of drinking milk when using different types of additives in different concentrations increases, with the highest value was found at 0.3% basil.

Saturated fatty acids in the milk control group were 78.92 g/100 g fat. The use of a basil supplement lowers their content, and the lowest values were reported at 0.1% supplement – 71.61 g/100 g fat. Lemon balm was lowered the saturated fatty acid content of drinking milk at 0.05% supplementation to 74.25 g/100 g fat compared to control milk, and increased at 0.1 and 0.2% supplementation. Addition of peppermint and thyme led to a decrease in the concentration of saturated fatty acids with increasing amount of supplementation and the lowest values were reported at 0.2%, 72.86 and 71.98 g/100 g fat, respectively. Saturated fatty acids were lowest at 0.1% rosemary supplementation and at 0.3% turmeric with black pepper. The application of all types of additives in different concentrations in milk leads to a decrease in saturated fatty acids, but the best results were obtained with the use of peppermint and thyme.

Monounsaturated fatty acids in the milk control group were 18.89 g/100 g fat. Adding basil to drinking milk increased their content at 0.05, 0.1 and 0.2% supplementation, and decreased to 16.96 g/100 g fat at 0.3% compared to control milk. Lemon balm in a low concentration leads to an increase in monounsaturated fatty acids and with increasing concentration they decrease to 17.68 g/100 g fat. The use of peppermint, thyme, rosemary and the combination of turmeric and black pepper increased the amount of monounsaturated fatty acids as follows for 0.2% peppermint – 21.84 g/100 g fat, 0.1% thyme – 22.83 g/100 g fat, 0.1% rosemary- 21.98 g/100 g fat and 0.3% turmeric with black pepper- 36.38 g/100 g fat. The application of supplements of different types of aromatic plants increased the content of monounsaturated fatty acids and the best result obtained with 0.3% turmeric with black pepper.

Polyunsaturated fatty acids in whole cow's milk were

Table 3. Physicochemical composition of drinking milk with additives, n = 10

	K		B1		B2		B3		B4		M1		M2		M3		P1		P2		P3	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
F	3.24 ^{GTHQN*}	0.05	3.36	0.10	3.42	0.11	3.61	0.18	3.95	0.51	3.51	0.02	3.69	0.02	3.66	0.11	3.41 ^{HIJ}	0.06	3.26	0.01	3.24^{HIJ}	0.06
D	33.48^{P}	0.66	33.91	0.01	32.04	3.06	30.18	2.42	28.74	0.10	29.11	0.19	28.84	0.09	28.99	0.24	32.84^{HJ}	0.14	34.02	0.13	34.21	0.37
C	4.72^{BCDEFGHIJLMNT}	0.09	4.81	0.19	5.06	0.09	5.00	0.11	4.97	0.03	5.01	0.01	4.94	0.02	4.97	0.02	5.01 ^{HJ}	0.08	5.10	0.03	5.17	0.05
S	9.58^{M}	0.17	9.63	0.15	9.24	0.79	8.67	0.75	8.42	0.03	8.49	0.04	8.46	0.02	8.51	0.06	9.45^{IJ}	0.05	9.73	0.04	9.78	0.10
P	3.50	0.06	3.52	0.05	3.37	0.29	3.43	0.33	3.07	0.01	3.10	0.02	3.08	0.01	3.10	0.02	3.45	0.02	3.56	0.01	3.55	0.06
W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L	5.26^{EP}	0.10	5.29	0.08	5.07	0.43	4.82	0.34	4.07	0.93	4.66	0.02	4.64	0.01	4.67	0.02	5.19^{HJ}	0.03	5.34 ^{IK}	0.02	5.37	0.06
T	22.00^{ABDFGKL}	2.29	24.50 ^{AD}	2.50	27.10 ^{BD}	0.80	29.12	2.79	30.65	0.15	30.23	0.25	30.37	0.40	30.47	0.31	26.03	1.21	26.73	0.40	27.03	0.21
FP	0.62	0.01	0.62	0.01	0.59	0.05	0.59	0.05	0.54	0.00	0.54	0.00	0.54	0.00	0.54	0.00	0.61 ^{HJ}	0.00	0.63 ^{IK}	0.00	0.63	0.01
S	0.78^{FGP}	0.02	0.78	0.01	0.75	0.06	0.76	0.07	0.94	0.35	0.69	0.01	0.69	0.00	0.69	0.00	0.77^{HI}	0.01	0.79^{IK}	0.00	0.80	0.01
pH	6.47^{P}	0.02	6.47	0.01	6.48	0.02	6.42	0.19	6.75	0.24	6.45	0.02	6.44	0.03	6.46	0.00	6.48^{HJ}	0.01	6.48	0.02	6.46	0.01
Fibber	0.00	0.00	1.89	0.24	3.72	0.27	7.42	0.46	11.15	0.29	1.64	0.34	3.35	0.34	6.64	0.27	1.44	0.19	2.93	0.11	5.94	0.48
E	64.26	1.10	69.27 ^{AD}	0.73	71.96 ^{BC}	2.57	80.37	0.70	86.38	6.57	65.93	0.68	70.77	0.69	77.33	0.83	68.09 ^{HI}	1.04	70.82	0.46	76.73	1.94

F – fat, %; D-density, °G; C – conduction, mS/cm; S – soluble non-fat (SNF),%; P – protein, %; W – water addition,%; L – lactose, %; FP- freezing temperature, (– °C); S – salt (ash), %; pH – active acidity; E – energy, kcal; * Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance

Table 3. Physicochemical composition of drinking milk with additives, n=10 (continue)

	T1		T2		T3		R1		R2		R3		C1		C2		C3		C4	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
F	3.53	0.06	3.58	0.04	3.51	0.03	3.35	0.09	3.46	0.05	3.84	0.04	3.39^{QRS}	0.05	3.29^{QR}	0.03	3.26^{QS}	0.01	3.40	0.02
D	28.75	0.11	28.71	0.08	28.13	0.78	33.24	0.62	29.74	1.99	30.69	0.14	33.78^{QST}	0.13	33.83^{RT}	0.13	34.03^{ST}	0.06	33.15	0.20
C	4.92^{KL}	0.02	4.97	0.02	4.93	0.07	5.00	0.09	5.03	0.04	5.40	0.04	4.74^{QST}	0.04	4.79^{RT}	0.07	4.89^{ST}	0.05	4.97	0.07
S	8.40	0.04	8.40	0.01	8.57	0.38	9.54	0.14	9.01	0.59	8.75	0.05	9.70^{QT}	0.03	9.68^{RT}	0.03	9.74^{ST}	0.02	9.54	0.06
P	3.06	0.01	3.27	0.37	3.00	0.08	3.49	0.06	3.29	0.22	3.20	0.02	3.54^{QT}	0.01	3.54^{RT}	0.01	3.56^{ST}	0.01	3.48	0.02
W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
L	4.68	0.10	4.61	0.01	4.52	0.12	5.27	0.07	4.95	0.33	4.81	0.03	5.32^{QT}	0.02	5.32^{RT}	0.02	5.34^{ST}	0.01	5.23	0.03
T	29.90	0.35	30.30	0.26	29.77	1.63	25.81^{NOP}	0.63	27.43	0.42	30.70	1.39	23.30^{QT}	0.80	24.03^{RT}	0.49	24.67	0.47	25.77	0.61
FP	0.53	0.00	0.53	0.00	0.52	0.02	0.61^{NP}	0.01	0.58	0.04	0.56	0.01	0.62	0.00	0.62^{RS}	0.00	0.63	0.00	0.61	0.00
Salt	0.68	0.00	0.68	0.00	0.71	0.08	0.78	0.01	0.73	0.05	0.71	0.00	0.79^{QT}	0.00	0.76	0.05	0.79^{ST}	0.00	0.78	0.01
pH	6.48	0.01	6.47	0.01	6.47	0.02	6.48^{NP}	0.01	6.48	0.00	5.68	0.16	6.47^{QT}	0.01	6.56	0.17	6.48	0.01	6.49	0.01
Fiber	1.83	0.17	3.71	0.17	7.41	0.42	2.19	0.34	4.26	0.37	8.49	0.50	1.25	0.09	2.29	0.24	4.67	0.21	6.97	0.25
E	66.40	1.35	71.14	2.21	76.47	1.90	69.57^{NP}	0.69	72.64	1.17	83.53	0.65	68.50	0.74	69.61	0.85	74.30	0.53	79.42	0.91

F – fat, %; D-density, °G; C – conduction, mS/cm; S – soluble non-fat (SNF),%; P- protein, %; W – water addition, %; L – lactose, %; FP- freezing temperature, (– °C); S – salt (ash), %; pH – active acidity; E – energy, kcal; * Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance

2.44 g/100 g fat. The addition of 0.2 and 0.3% basil increased their content compared to the control milk to 2.56 and 3.15 g/100 g fat. The use of lemon balm lowers the content of polyunsaturated fatty acids compared to the control milk, and at 0.1% addition was 2.18 g/100 g fat. Polyunsaturated fatty acids have the highest content at 0.1% peppermint – 2.75 g/100 g fat, 0.05% thyme – 2.82 g/100 g fat, 0.1% rosemary – 2.92 g/100 g fat and 0.3% turmeric – 4.35 g/100 g fat. The total content of biologically active trans fatty acids in the control milk was 4.70 g/100 g fat. The main representatives of fatty acids in basil were stearic, oleic, palmitic, linoleic, myristic, α-linolenic, capric, lauric and arachidonic acids (Shahrajabian et al., 2020).

The introduction of the different supplements in three or four concentrations leads to their change, which is probably caused by oxidation processes to increase their content, but in general case, they decrease due to the introduction of the plant additive, which mainly contains cis isomers of oleic acid. The biologically active cis isomers of oleic acid in the source milk have a 11.24 g/100 g fat content. The use of peppermint (0.2% – 15.70 g/100 g fat), thyme 0.2% – (15.20 g/100 g fat), rosemary (0.1% – 16.05 g/100 g fat), and the combination of turmeric with black pepper (0.3% – 28.92 g/100 g fat) increased the content of cis isomers from oleic acid. Biologically active omega-3 and omega-6 fatty acids in the studied milk were 0.43 g/100 g fat and 1.70 g/100 g fat, respectively. Adding the plant supplements in different concentrations leads to a decrease in omega-3 with the exception of 0.3% turmeric with black pepper – 0.52 g/100 g fat. Omega-6 fatty acids, when adding basil vary depending on the concentration, and have the highest value at 0.2% – 2.08 g/100 g fat. The use of 0.1% lemon balm has the lowest concentration of omega-6 fatty acids in the group and compared to control milk – 1.63 g/100 g fat. The addition of 0.2% peppermint-1.85 g/100 g fat and thyme-1.95 g/100 g fat were the lowest in the respective group, but higher than the control milk. The use of rosemary and turmeric increased the content of omega-6 fatty acids in drinking milk. At 0.1% rosemary – 2.25 g/100 g fat and 0.3% the combination of turmeric with black pepper – 3.60 g/100 g fat the highest values were found. The use of additives from plant origin in different concentrations in drinking milk led to a slight decrease in the content of medium-chain fatty acids, while in short-chain fatty acids were from 2 to 43 times (Table 4).

The lipid preventive score in the studied milk from the control group was 7.82. The addition of 0.2% lemon balm increased it in milk to 8.31, 0.5% peppermint – 8.13, 0.2% rosemary – 8.04 and 0.3% turmeric with black pepper – 8.06. The atherogenic index in milk was 4.35, and the thrombo-

Table 4. Fatty acid groups in drinking milk with additives, g/100 g fat, n = 10

	K		B1		B2		B3		B4		M1		M2		M3	
SFA	78.92	0.13	75.99	0.05	71.61	2.33	72.05	0.51	74.17	0.03	74.25	0.07	76.78	4.43	77.39	2.63
MUFA	18.89	0.03	21.40	0.02	20.24	0.66	20.85	1.68	16.96	8.99	20.85	0.02	19.83	0.72	17.68	0.17
PUFA	2.44	0.01	2.32	0.00	2.28	0.07	2.56	0.45	3.15	0.00	2.34	0.00	2.18	0.08	2.35	0.02
Σ C-18:1Trans-FA	4.70	0.01	4.76	0.00	3.47	0.11	7.25	1.25	2.86	0.00	4.90	0.00	4.06	0.15	3.52	0.03
ΣCLA	0.41	0.00	0.24	0.00	0.29	0.01	0.21	0.05	0.21	0.00	0.22	0.00	0.21	0.01	0.28	0.00
C-16:0/C-18:1cis9	3.62	0.00	3.20	0.00	3.02	0.00	3.95	0.09	2.60	0.00	3.40	0.00	3.40	0.00	3.88	0.00
C-16:0/C-18:1 ges.	2.47	0.00	2.29	0.00	2.38	0.00	2.26	0.12	5.38	5.62	2.38	0.00	2.53	0.00	2.86	0.00
Σn-3	0.43	0.01	0.35	0.00	0.30	0.01	0.37	0.05	0.40	0.00	0.36	0.00	0.33	0.01	0.28	0.00
Σn-6	1.70	0.00	1.83	0.00	1.75	0.06	2.08	0.48	2.65	0.00	1.86	0.00	1.63	0.06	1.86	0.02
ΣMCT(C-10>C-14)	20.61	0.03	18.63	0.01	19.48	0.63	17.32	1.09	18.88	0.01	19.42	0.02	19.52	0.71	18.80	0.18
ΣSCT(C-4>C-8)	7.68	0.01	4.97	0.00	0.22	0.01	3.44	1.00	4.98	0.00	0.94	0.00	4.20	7.05	4.35	2.81
CLA 9c,11t	0.26	0.00	0.06	0.00	0.11	0.00	0.06	0.01	0.07	0.00	0.07	0.00	0.07	0.00	0.12	0.00
Σn-6/Σn-3	4.00	0.10	5.20	0.00	5.74	0.00	5.89	2.07	6.65	0.00	5.23	0.00	4.96	0.00	6.66	0.00
Σ C-18:1cis-FA	11.24	0.02	13.41	0.01	13.79	0.45	10.64	0.27	10.93	8.99	12.94	0.01	12.63	0.46	11.49	0.11
BFA	3.09	0.00	3.11	0.00	3.55	0.12	3.37	0.04	3.37	0.00	3.15	0.00	3.52	0.13	3.27	0.03

Table 4. Fatty acid groups in drinking milk with additives, g/100 g fat, n = 10 (continue)

	P1		P2		P3		T1		T2		T3	
SFA	73.55	0.04	73.83	0.05	72.86	0.04	73.28	0.04	72.49	0.05	71.98	0.04
MUFA	20.94	0.01	20.79	0.01	21.84	0.01	22.52	0.01	22.83	0.01	22.09	0.01
PUFA	2.51	0.00	2.75	0.00	2.34	0.00	2.82	0.00	2.69	0.00	2.60	0.00
Σ C-18:1Trans-FA	3.05	0.00	6.49	0.00	2.84	0.00	3.99	0.00	3.77	0.00	3.42	0.00
ΣCLA	0.31	0.00	0.41	0.00	0.17	0.00	0.27	0.00	0.27	0.00	0.30	0.00
C-16:0/C-18:1cis9	2.96	0.00	3.84	0.00	2.78	0.00	3.05	0.00	3.01	0.00	2.81	0.00
C-16:0/C-18:1 ges.	2.39	0.00	2.35	0.00	2.30	0.00	2.31	0.00	2.31	0.00	2.25	0.00
Σn-3	0.35	0.00	0.35	0.00	0.38	0.00	0.41	0.00	0.34	0.00	0.40	0.00
Σn-6	1.95	0.00	2.09	0.00	1.85	0.00	2.24	0.00	2.18	0.00	1.95	0.00
ΣMCT(C-10>C-14)	20.24	0.01	19.61	0.01	18.74	0.01	20.74	0.01	20.17	0.01	18.20	0.01
ΣSCT(C-4>C-8)	0.12	0.00	1.34	0.00	0.18	0.00	1.32	0.00	0.07	0.00	0.26	0.00
CLA 9c,11t	0.12	0.00	0.17	0.00	0.00	0.00	0.10	0.00	0.10	0.00	0.06	0.00
Σn-6/Σn-3	5.57	0.00	5.93	0.00	4.87	0.00	5.45	0.00	6.40	0.00	4.82	0.00
Σ C-18:1cis-FA	14.67	0.01	11.45	0.01	15.70	0.01	14.17	0.01	14.68	0.01	15.20	0.01
BFA	3.50	0.00	3.44	0.01	3.32	0.00	2.20	0.00	2.29	0.00	3.61	0.00

genic index was 3.85. The application of thyme, rosemary and the combination of turmeric with black pepper decreased the atherogenic and thrombogenic index in milk at all concentrations used (Table 5). The atherogenic index gives the correlation between the sum of the main saturated fatty acids and the unsaturated fatty acids, the former being considered proatherogenic (favouring the adhesion of lipids in the cells of the immune and circulatory system), and the second were antiatherogenic (inhibit plaque aggregation and decrease levels of esterified fatty acids, cholesterol and phospholipids, thus preventing the occurrence of micro- and macrocoronary diseases). The thrombogenic index has the tendency to clot formation in blood vessels and has defined as the ratio between prothrombogenic (saturates) and antithrombogenic (monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids) fatty acids (Ghaeni et al., 2013). The thrombogenic and atherogenic index, as indicators, should not exceed 1.00, while the cholesterol index is above 1.00 (Ivanova and Hadzhinikolova, 2015). The hyper-hypocholesterolemic index for milk was 0.25 and increases slightly, when the additives were added with different concentrations. Use of 0.3% basil increased h/H to 0.84, 0.05% lemon balm to 0.35, 0.1% peppermint to 0.63, 0.05% thyme to 0.32, 0.05% rosemary to 1.80 and 0.2% turmeric with black pepper -0.77. Cholesterolemic index was low (below 1.0) in all variants of supplements and concentrations except for 0.05%

Table 4. Fatty acid groups in drinking milk with additives, g/100 g fat, n = 10 (continue)

	R1		R2		R3		C1		C2		C3		C4	
SFA	73.39	0.28	72.14	0.51	76.07	0.05	75.59	0.04	75.03	3.41	72.10	0.04	56.97	0.85
MUFA	21.61	0.08	21.98	0.01	20.83	0.01	20.99	0.01	20.20	0.41	21.45	0.01	36.38	0.02
PUFA	2.51	0.01	2.92	0.00	2.66	0.00	2.53	0.00	2.53	0.05	2.83	0.00	4.35	0.00
Σ C-18:1Trans-FA	3.47	0.01	2.71	0.00	4.34	0.00	4.07	0.00	2.76	0.06	2.53	0.00	4.51	0.00
ΣCLA	0.23	0.00	0.29	0.00	0.27	0.00	0.32	0.00	0.33	0.01	0.29	0.00	0.43	0.00
C-16:0/C-18:1cis9	2.80	0.00	2.66	0.00	3.12	0.00	3.05	0.00	2.86	0.00	2.63	0.00	0.98	0.00
C-16:0/C-18:1 ges.	2.23	0.00	2.22	0.00	2.29	0.00	2.31	0.00	2.37	0.00	2.21	0.00	0.81	0.00
Σn-3	0.40	0.00	0.44	0.00	0.36	0.00	0.40	0.00	0.37	0.01	0.38	0.00	0.52	0.00
Σn-6	1.95	0.01	2.25	0.00	2.11	0.00	1.85	0.00	1.85	0.04	2.24	0.00	3.60	0.00
ΣMCT(C-10>C-14)	18.90	0.07	19.30	0.01	20.20	0.01	20.10	0.01	20.65	0.28	20.33	0.01	11.49	0.01
ΣSCT(C-4>C-8)	2.16	0.01	0.60	0.05	4.84	0.00	4.12	0.00	3.69	0.92	1.36	0.00	1.35	0.83
CLA 9c,11t	0.06	0.00	0.08	0.00	0.11	0.00	0.16	0.00	0.15	0.00	0.14	0.00	0.21	0.00
Σn-6/Σn-3	4.89	0.00	5.17	0.00	5.93	0.00	4.62	0.00	4.97	0.00	5.88	0.00	6.92	0.00
Σ C-18:1cis-FA	14.91	0.06	16.05	0.00	13.37	0.01	13.61	0.01	14.20	0.29	15.68	0.01	28.92	0.02
BFA	3.45	0.01	3.23	0.00	3.17	0.00	3.41	0.00	3.26	0.07	3.21	0.00	2.85	0.00

rosemary. Trans fatty acids in the control group of milk were 0.15 g/100 g product, and vary when adding aromatic plants in different concentrations, with the lowest values found at 0.3% basil – 0.03 g/100 g product, 0.1 % peppermint- 0.4 g/100 g product, 0.05% thyme-0.10 g/100 g product, 0.2% rosemary-0.10 g/100 g product and 0.2% turmeric with black pepper-0.02 g/100 g product. The introduction of the additives from different aromatic plants leads to an improvement of the fatty acid profile and the quality indicators of the drinking milk compared to the control milk.

Antioxidants can delay or inhibit oxidative damage to proteins, nucleic acids and lipids, caused by free radicals that induce oxidative stress (Baardseth, 1989; Norshazila et al., 2010; Zheng and Wang, 2001) through autoxidation. Lipid oxidation leads to undesirable changes in the taste, texture and nutritional value of foods (Wang et al., 2006). Milk proteins were characterized by scavenging ability of active oxygen species or free radicals (Hambraeus and Lönnerdal, 2003; Karakaya et al., 2001; Lindmark-Månsson and Åkesson, 2000; Suetsuna et al., 2000; Wang et al., 2006), and were used as natural antioxidants in the food industry due to the fact, that they have no taste and smell, but were nutritious. Phenolic compounds were widely present in dairy products, but literature data regarding their study in milk and dairy foods is scarce. Ertan et al. (2017) were found an increase in antioxidant activity and polyphenol content in milk with increasing fat content in UHT-milk. According to Calligaris et al. (2003), the application of different temperature regimes for pasteurization of milk increased the antioxidant activity (Table 6).

The antioxidant activity in the studied milks was 4.16 TE mg/100 g product. Milk with additives was characterized by different antioxidant activity, depending on their type and concentration. Adding 0.05 and 0.1% basil to milk increased antioxidant activity by 3.8 times, 0.2% by 4.9 times, and 0.3% by 6.6 times compared to control milk. The use of lemon balm increased the antioxidant activity of milk by 2.6 times at 0.1%, by 3.3 times at 0.05% and by 3 times at 0.2% compared to control milk. Addition of peppermint to milk leads to an increase in antioxidant activity with increasing

Table 5. Qualitative evaluation of the fat fraction of drinking milk with supplements

	K	B1	B2	B3	B4	M1	M2	M3	P1	P2	P3	T1	T2	T3	R1	R2	R3	C1	C2	C3	C4
LPS	7.82	7.92	7.37	7.78	3.74	7.70	7.84	8.31	8.13	3.52	7.80	7.95	8.10	7.75	3.52	7.99	8.04	7.70	7.85	3.60	8.06
AI	4.35	3.90	4.18	4.13	1.39	4.63	4.16	4.39	4.38	1.70	4.15	3.94	3.84	3.97	1.77	3.85	3.85	4.03	3.91	2.62	3.91
TI	3.85	3.49	3.52	3.61	1.32	3.44	3.64	3.81	3.63	1.63	3.47	3.59	3.33	3.46	1.30	3.49	3.39	3.33	3.40	2.66	3.24
h/H	0.25	0.28	0.29	0.27	0.84	0.35	0.26	0.26	0.29	0.63	0.24	0.32	0.29	0.28	1.80	0.32	0.33	0.29	0.31	0.77	0.33
TFA, g/100 g product	0.15	0.16	0.12	0.14	0.03	0.10	0.17	0.14	0.14	0.04	0.22	0.10	0.15	0.16	0.06	0.12	0.10	0.15	0.12	0.02	0.09
SFA+TFA, g/100 g product	2.75	2.79	2.49	2.68	0.16	2.58	2.74	2.89	2.74	0.17	2.78	2.70	2.81	2.76	0.12	2.74	2.72	2.69	2.72	0.07	2.72

Table 6. Antioxidant activity and content of total polyphenols in drinking milk with supplements, n=10

	DPPH, TE mg/100 g product	TPC, GAE mg/100 g product
K	0.92±0.02^{KL*}	23.33±0.57^{E*}
B1	3.48±0.07^{D}	12.50±0.01^{K}
B2	3.43±0.119^{D}	15.83±0.28^{J}
B3	4.50±0.26^{C}	19.33±0.57^{GHI}
B4	6.05±0.31^{B}	21.83±0.57^{EF}
M1	2.99±0.49^{DE}	18.50±0.50^{I}
M2	2.37±0.00^{FGH}	28.50±0.00^{D}
M3	2.83±0.02^{EF}	19.17±0.29^{HI}
P1	1.02±0.16^{K}	21.17±0.28^{FG}
P2	1.40±0.08^{JK}	20.00±0.00^{FGHI}
P3	1.91±0.17^{HIJ}	40.00±1.73^{B}
T1	2.18±0.21^{GHI}	30.33±1.443^{CD}
T2	1.72±0.17^{IJ}	21.00±0.500^{FGH}
T3	2.54±0.00^{EFG}	30.83±0.57^{C}
R1	0.87±0.05^{KL}	6.00±0.001^{L}
R2	1.94±0.11^{HI}	7.67±0.289^{L}
R4	4.63±0.012^{C}	14.01±0.058^{JK}
C1	0.42±0.00^{L}	6.01±0.010^{L}
C2	2.20±0.04^{GHI}	20.83±0.289^{FGH}
C3	4.43±0.15^{C}	45.17±0.289^{A}
C4	7.19±0.15^{A}	38.17±0.289^{B}

*Means not sharing any letter are significantly different by the Tukey-test at the 5% level of significance

concentration compared to control milk, up to 1.02, 1.40 and 1.90 TE mg/100 g product, respectively. Adding thyme to milk increased the antioxidant activity of milk by 2.4 times at 0.05%, by 1.9 times at 0.1% and by 2.8 times at 0.2%. The application of 0.05% rosemary in drinking milk decreased the antioxidant activity compared to the control milk, but with increasing concentration to 0.1% it increased twofold, and at 0.2% fivefold. The use of 0.05% turmeric with black pepper decreased the antioxidant activity of milk twofold, but with increasing concentration it increased by 2.4 times at 0.1%, by 4.8 times at 0.2% and by 7.8 times at 0.3%. All types of additives led to an improvement in the antioxidant activity of their milk group, except for 0.05% rosemary and 0.5% turmeric with black pepper, and the highest values were obtained at the higher concentrations from combination of turmeric and black pepper.

The total polyphenols in the studied milk were 23.33 GAE mg/100 g product. The addition of basil lowers the content of polyphenols at all selected concentrations. In lemon balm, a higher amount of total polyphenols was reported at 0.1% supplementation, while at 0.05 and 0.2%, they were lower compared to the control milk. The application of 0.05 and 0.1% peppermint in milk had a lower content of polyphenols compared to the control milk, and increased when used 0.2% additive to 40 TE mg/100 g product (1.7 times). Thyme increased the content of polyphenols in milk at 0.05% to 30.33 TE mg/100 g product and at 0.2% to 30.83 TE mg/100 g product compared to control milk. Rosemary addition to milk decreased total polyphenol content at all three concentrations compared to control milk. The use of 0.05 and 0.1% turmeric with black pepper decreased the content of polyphenols, while at 0.2 and 0.3% it increased compared to control milk to 45.17 and 38.17 TE mg/100 g product. The highest content of polyphenols in milk with additives was found with 0.2% peppermint – 40.00 TE mg/100 g product and 0.2% turmeric with black pepper – 45.17 TE mg/100 g product.

A number of studies on the incorporation of essential oils leads to the preservation of milk and dairy products and prolongation of their shelf life (Bukvicki et al., 2018; Caleja et al., 2015; Hala et al., 2010; Mohamed et al., 2018).

Conclusions

Adding supplements of aromatic plant in different concentrations improves the qualitative and quantitative composition of milk. The organoleptic evaluation of the studied milk gives us a clear idea, that the application of a higher concentration of additives worsens its smell, taste, colour, aroma and texture, but enriches it with fibbers, minerals and fats, and as a result of them the energy value also increases. Drinking milk with the addition of aromatic plants in dry form has a reduced content of saturated fatty acids and is enriched with biologically active fatty acids, which in turn leads to health benefits from their consumption and was expressed by lowering AI and TI and increasing cholesterolemic index. The antioxidant activity of milk was improved and the total content of polyphenols was increased, with the highest values obtained when adding turmeric with black pepper.

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