Effect of the addition of turmeric and black pepper essential oil on the composition of curd

Silviya Ivanova[1*], *Ivan Kostov*[1], *Georgi Angelov*[1], *Nadejda Daskalova*[1]

[1] Institute of Cryobiology and Food Technology, Agricultural Academy, Department of Food Technology, Sofia 1407, Bulgaria

Abstract. The aim of the study was to determine the physicochemical and biochemical composition of cow's milk curd obtained after technological processing with the addition of turmeric and black pepper essential oil /3:1 ratio/ in different concentrations. Six concentrations were tested to determine the optimal dose of the additive. The changes were determined by evaluating the organoleptic, physicochemical and biochemical indicators at 24 h and after storage in refrigerated conditions for 3, 7, 14 and 21 days. The total fat content was highest when adding 2 and 2.5% turmeric and black pepper essential oil at 24 h compared to the control curd ($P \leq 0.001$). The protein did not vary significantly both at 24 h and when monitoring its change during refrigerated storage for up to 21 days. The application of turmeric and black pepper essential oil in different concentrations improves the antioxidant capacity and total polyphenol content, both depending on the concentration and the shelf life. The use of the additive led to an increase in saturated fatty acids at 24 h and on day 3 at the expense of a decrease in unsaturated fatty acids, but on 7th and 14th days saturated fatty acids decrease and increase again on 21st days.

1 Introduction

Functional food products have been the most promising area in the dairy industry in the last decade. Products of this type take into account the balanced nutrition of different population groups and are able to satisfy the physiological needs of energy and essential substances [9, 14, 15].

The combination of turmeric and black pepper in warm milk was recommended for sore throats, coughs, colds and other acute respiratory infections. Turmeric contains the lipophilic bioactive compound curcumin with antioxidant and anti-inflammatory properties [10]. Idowu-Adebayo et al. [11] studied the effect of turmeric addition to soy milk and hibiscus drink on organoleptic properties and consumer acceptability.

Idowu-Adebayo et al. [12] investigated the nutritional value and antioxidant activity of soy milk and hibiscus drink with turmeric addition. Idowu-Adebayo et al. [13] investigated the effect of adding turmeric paste to soy milk with and without heat treatment, which increased the nutritional and chemical value of all variants and reported an increase in protein, iron, zinc, TPC (total phenolic content) and antioxidant activity.

Dosoky and Setzer [1] reviewed the literature on turmeric essential oil and they found that its characterized by anti-inflammatory, anticancer, antiproliferative, hypocholesterolemic, antidiabetic, antihepatotoxic, antidiarrheal, carminative, diuretic, antirheumatic, hypotensive, antioxidant, antimicrobial, antiviral, insecticidal, larvicidal, antivenom and antithrombogenic properties.

Avanço et al. [2] found antioxidants, antifungal and antimycotoxigenic effects of C. longa essential oil on F. Verticillioides and the oil can be applied as a food preservative and would increase the shelf life of agricultural products. In addition, the oil can reduce lipid peroxidation and other processes mediated by post-harvest free radical formation.

Rodrigues et al. [6] in their literature review have presented black pepper essential oil as a reliable source for preserving and improving food quality, as it was characterized by antioxidant, antimicrobial and anti-inflammatory properties based on the content of the substance piperine, which inhibits the development of pathogenic microflora. They found an increase in the total content of saturated fatty acids depending on the added amount of black pepper essential oil in chicken pâté and a variation in the content of mono and polyunsaturated fatty acids compared to the control group.

The aim of the study was to determine the physicochemical and biochemical composition of cow's milk curd obtained after technological processing with the addition of turmeric and black pepper essential oil /3:1 ratio/ in different concentrations for 24 hours and during refrigerated storage for 3, 7, 14 and 21 days.

2 Material and methods

Milk collected from highly productive Bulgarian Rhodope cattle was used, taken every month for one year from the Research Centre of Stockbreeding and

* Corresponding author: sylvia_iv@abv.bg

Agriculture, Smolyan. The milk was pasteurized (4 litters) and technological processing into curd (Figure 1) was carried out, adding supplements from turmeric and black pepper pre-mixed in a 3:1 ratio and added in the following concentrations: 0% - control /K/ and 0.5%, 0.75%, 1 %, 1.5%, 2% and 2.5%. Six different batches of collected milk were used to produce curd - control group and with the addition of curcuma and black pepper oil in different concentrations. The essential oils – turmeric and black pepper were obtained by the steam extraction method and were purchased from Budjak Ltd. The technological scheme is presented in Figure 1.

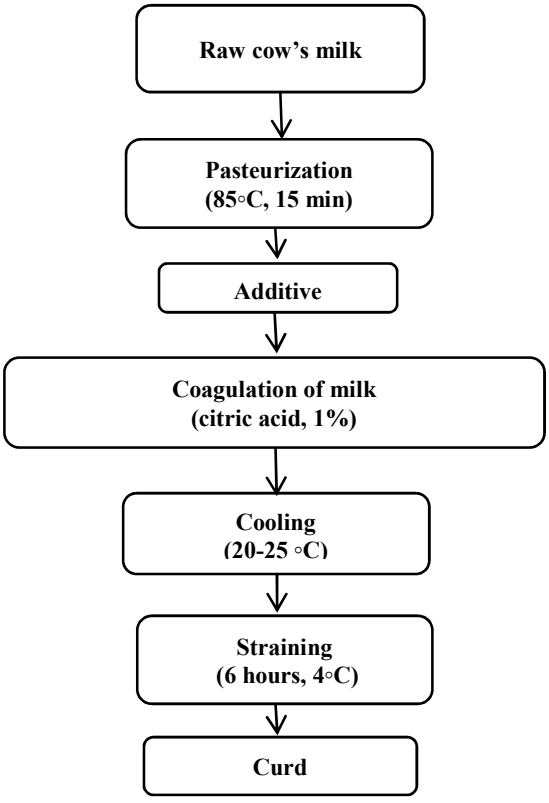

Fig. 1. Technological scheme for obtaining a curd

The study was conducted with technological milk every other month, to establish changes after technological processing, caused by the quality of the milk and to monitor the changes in organoleptic, physicochemical and biochemical indicators at 24 h, 3, 7, 14 and 21 days. An organoleptic assessment of curd without and with an additive in different concentrations was performed to determine the most appropriate concentration based on the sensory perceptions of consumers (10 panellists) and was conducted, according to the guidelines for ethics and food research set by the European Union [19] during the storage. The appearance, texture, smell, aroma and taste were examined with ratings from 1 to 5, where 1 was extremely disliked and 5 was extremely liked.

 Total protein- ISO 9622, BDS EN ISO 8968-1:2002 [21]

 Fat- BDS EN ISO 1211:2002, ISO 9622 [22]

 pH- with pH- meter model MW102-FOOD

 Total lipids were extracted using the Roese & Gottlieb method [23]. Fatty acid methyl esters /*FAME*/ were analysed using a Shimadzu-2010 gas chromatograph (Kyoto, Japan).

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

The curd was extracted with 95% ethanol (Merck) at a sample: extractant ratio of 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 № 4 paper) were stored at -20°C for subsequent analyses.

Determination of total phenolic content in curd

To quantify the total phenolic content (TPC), the method of Singleton et al. [16] was used with modification of Valyova et al. [17]. Briefly, 3.0 ml of distilled water and 0.25 ml of Folin–Ciocalteu reagent (Roth) were added to 0.5 ml of the sample (with the appropriate dilution). After standing for 2 minutes, 0.75 ml of 20% sodium carbonate solution (Merck) and 0.5 ml of distilled water were added to the mixture. The 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 minutes. TPC was calculated using the standard gallic acid line and expressed as milligrams of gallic acid equivalents per 100 grams of fresh product (mg GAE/100 g product).

Determination of antioxidant activity

The antioxidant capacity of the samples was assessed by determining the 1,1-diphenyl-2-picrylhydrazyl (DPPH-Merck) radical scavenging capacity according to the method of Brand-Williams et al. [18] with slight modification: 0.6 mL of a 0.2 mM solution of DPPH in methanol was mixed with 0.9 mL of methanol (Merck) and 0.5 mL of the corresponding sample dilution. The absorbance was measured after standing (60 minutes) 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 with 0.5 mL of 80% methanol. The antioxidant activity was calculated against the Trolox standard curve and the results were expressed as milligrams of Trolox equivalents per 100 grams of fresh product – mg TE/100 g product.

All solvents and standards were of high purity – ultra pure (99.99%).

The results were processed using the statistical package of the computer program EXCEL 2016. The reliability of the differences between the studied cheeses was established using the Student's t-test.

3 Results and discussion

The organoleptic study conducted by consumers provides information about the change in five indicators, with all indicators being most acceptable at concentrations of when using 0.5; 0.75% and 1% additive, and the lowest-rated samples were those with 2.0% and 2.5% supplement in all indicators compared to the control sample and the remaining concentrations at shelf life period.

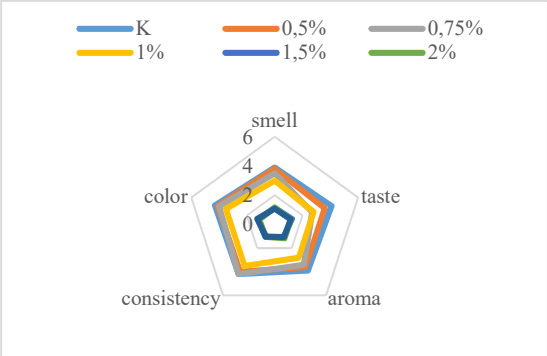

Fig. 2. Organoleptic evaluation of curd with the addition of turmeric and black pepper essential oil 3:1; 1-unacceptable; 2-acceptable; 3-I like it; 4- I really like it; 5-I like it very much

Ivanova et al. [20] in the study of cow's milk curd obtained a total protein content of 12.16% and when using the turmeric and black pepper dry additive at 0.05 and 0.1%, a loss of protein was found, 1 and 2% respectively, while at higher concentrations - 0.2 and 0.3% of them it was preserved and even increased. The supplementation of the add-on leads to an increase in the fat content, which on the one hand was caused by the fat concentration in the added ingredient, and on the other hand to the preservation of the fat after technological processing in the curd itself and isn't separated with the whey.

Table 1. Protein content in curd by addition of turmeric and black pepper essential oil 3:1, (n=6)

P, %	24h		3d		7d		14d		21d	
	X	SD	X	SD	X	SD	X	SD	X	SD
K	15.02 abcd	0.06	17.53	0.42	16.84	0.11	16.88	0.25	17.64	0.13
0.5%	15.40	0.04	15.25	0.24	16.71	0.27	16.90	0.18	17.02	0.01
0.75%	13.70	0.16	15.81	0.18	16.41	0.02	15.26	0.01	15.87	0.30
1%	16.83	0.04	15.86	0.41	16.82	0.11	16.91	0.42	16.21	0.07
1.5%	17.04 e	0.04	15.06	0.18	17.37	0.11	18.05	0.30	17.56	0.62
2%	15.18	0.15	14.27	0.13	16.39	0.13	15.48	0.17	14.38	0.16
2.5%	16.01	0.44	16.89	0.16	17.87	0.55	16.76	0.24	13.07	0.25

a- 24h/ 3d; b- 24h/ 7d; c- 24h/ 14d; d- 24h/ 21d; e- 24h K/ 24h 1.5%; P≤0.001

The analysed cow's milk curd had a 15.02% protein at 24 h and during storage its amount increased at 3 and 21 days to 17.53 and 17.64%, while at 7 and 14 days it was lower, respectively 16.84 and 16.88% (P≤0.001). The addition of turmeric with black pepper essential oils (TBPEO) significantly increased (P≤0.001) its content at 24h, with the highest concentration being found at 1.5%. The amount of protein when using a different concentration of the supplementation was significantly lower on the third day compared to the control group and the highest content was found at 2.5% add-on - 16.89%. The use of 1.5% TBPEO resulted in the highest protein amount in the curd at 7, 14 and 21 days rival to the control, which may be due to the retention capacity and a decrease in technological losses with whey separation. Our previous studies [20] on the amplification of turmeric and black pepper the dry substance show that the 0.2% (12.91%) and 0.3% (14.43%) additive leads to an increase in protein content measure up to the control group of curd - 12.16%.

The amount of fat in the studied curds varies, which is due to the additive used and the inhomogeneity of the studied samples, due to the absence of emulsifiers and stabilizers. The use of the supplement leads to an increase in the fat content (P≤0.001). This was due to the fat content of the additive and the fat remaining after technological processing in the curd itself and was not separated with the whey. In our previous studies [20], we used dry matter of turmeric and black pepper in different concentrations, the fat content was higher in supplementation curd from 8.96 to 13.52% compared to the control group- 5.9%. Storage and different concentrations of the added TBPEO increase the fat content in the curd (P≤0.001). The amount of fat was the lowest in all variants at 24 hours and ranges from 1.19% to 3.22% and the highest at 14 days of storage from 6.83 to 23.22%.

Table 2. Fat content in curd by supplement from turmeric and black pepper essential oil 3:1 (n=6)

F, %	24h		3d		7d		14d		21d	
	X	SD	X	SD	X	SD	X	SD	X	SD
K	1.19 abcdef	1.15	10.18	0.04	9.14	1.70	6.83	0.47	5.83	0.74
0.5%	2.62	0.15	6.34	1.92	3.51	1.51	23.22	4.09	5.55	2.29
0.75%	1.82	0.95	11.76	0.73	8.80	2.41	1.77	2.15	7.79	0.81
1%	2.23	0.41	14.15	0.57	4.96	2.89	14.67	2.58	7.12	0.79
1.5%	2.11	1.46	5.33	1.32	5.68	2.38	21.41	5.94	4.78	0.63
2%	2.77	0.03	5.84	0.60	6.18	1.54	2.85	0.16	4.32	1.71
2.5%	3.22	1.50	0.79	0.47	3.57	0.86	14.26	1.28	5.06	3.15

a-24h K/24h 0.5%; b- 24h K/24h 0.75%; c- 24h K/24h 1%; d- 24h K/24h 1.5%; e- 24h K/ 24h 2%; f-24h K/ 24h 2.5%; P≤0.001

The active acidity in control group curd was 5.41 at 24 h and after storage increased to 6.96 on 21st days. The use of the addition of turmeric and black pepper essential oil increased the pH, with the highest value being found at 1% - 5.60. Monitoring the change in active acidity showed a decrease in the curd with the supplement at 7 days and then increased to 21 days, but was significantly (P ≥ 0.001) lower than the control group curd (table 3).

Table 3. Active acidity in curd by addition of turmeric and black pepper essential oil 3:1, (n=6)

pH	24h		3d		7d		14d		21d	
	X	SD	X	SD	X	SD	X	SD	X	SD
K	5.41	0.13	5.53	0.01	5.65 abcdef	0.01	6.09 abcdef	0.03	6.96 abcdef	0.04
0.5%	5.56	0.04	5.73	0.07	5.18	0.11	5.49	0.09	5.89	0.13
0.75%	5.45	0.16	5.35	0.04	5.20	0.06	5.49	0.09	5.89	0.13
1%	5.60	0.02	5.48	0.07	5.05	0.04	5.43	0.20	5.84	0.06
1.5%	5.50	0.02	5.57	0.05	5.17	0.10	5.39	0.10	5.51	0.03
2%	5.54	0.05	5.49	0.02	5.33	0.03	5.42	0.12	5.90	0.10
2.5%	5.47	0.01	5.62	0.03	5.33	0.03	5.49	0.23	5.57	0.05

a- K/ 0.5%; b- K/ 0.75%; c- K/ 1%; d-K/ 1.5%; e- K/ 2%; f- K/ 2.5%; P≤0.001

The antioxidant activity (AA) of the studied curd was 8.90 mg TE/100 g product in the control group and varies depending on the concentration of the additive per

24 hours (table 4). When monitoring the changes in AA, the highest values in the control group curds and those with the addition of turmeric and black pepper essential oil were after 21 days of storage in refrigerated conditions. All variants of the additive increase an antioxidant activity except the 0.75%, where a lower value was found compared to the control group. In our previous studies on antioxidant activity, a lower value was found in the control group curds- 1.14 mg TE/100 g product and depending on the additive content by dry substance varied from 4.67 to 12.45 mg TE/100 g product [20].

Table 4. Antioxidant activity in curd by supplement from turmeric and black pepper essential oil 3:1 (n=6)

DPPH, mg TE/ 100 g product	24h		3d		7d		14d		21d	
	X	SD	X	SD	X	SD	X	SD	X	SD
K	8.90	0.03	11.53	0.05	10.58	0.08	14.97	0.09	41.50	0.05
0.50%	7.07	0.02	9.19	0.01	35.51	0.06	1.30	0.01	55.10	0.02
0.75%	5.39	0.01	8.09	0.01	12.92	0.03	16.72	0.04	6.19	0.01
1%	4.37	0.01	13.14	0.02	2.76	0.01	10.21	0.02	60.73	0.03
1.50%	29.07	0.09	13.07	0.02	8.53	0.02	44.50	0.08	57.66	0.09
2%	3.05	0.01	15.99	0.05	25.05	0.06	41.72	0.03	55.90	0.06
2.50%	19.72	0.09	31.56	0.08	22.35	0.09	5.90	0.02	48.01	0.09

Avanço et al. [2] found that the antioxidant activity of turmeric essential oil (EO) was most likely a result from synergy between its components. The major ingredients were responsible for the overall AA of the EO [4]. The essential oil of C. longa showed dose-dependent DPPH radical scavenging activity, indicating that the oil acts as a hydrogen donor antioxidant. The predicted IC50 value was 10.03 mg/ml which was satisfactory compared to the IC50 value of 4.5 mg/ml reported by Gounder and Lingamallu [5]. This difference was most likely due to the low percentage of ar-turmerone (12.9%), as reduced ar-turmerone reduces the DPPH radical scavenging activity of turmeric oil [5].

Table 5. Total polyphenols content in curd by addition of turmeric and black pepper essential oil 3:1, (n=6)

TPC, mg GAE/ 100 g product	24h		3d		7d		14d		21d	
	X	SD	X	SD	X	SD	X	SD	X	SD
K	68.92	0.09	33.35	0.05	38.64	0.05	51.67	0.08	55.37	0.05
0.5%	77.49	0.09	28.67	0.06	23.73	0.03	38.65	0.05	55.29	0.05
0.75%	23.80	0.03	28.03	0.06	36.34	0.05	34.45	0.05	56.30	0.06
1%	97.81	0.06	32.46	0.08	40.00	0.05	36.24	0.05	50.15	0.04
1.5%	94.48	0.08	35.54	0.08	59.24	0.06	81.11	0.09	78.72	0.08
2%	129.56	0.09	30.15	0.07	57.45	0.06	50.18	0.08	73.40	0.08
2.5%	154.79	0.09	150.00	0.09	57.50	0.06	97.97	0.09	122.18	0.09

The concentration of total polyphenols in the studied curd from the control group was 68.92 mg GAE/100 g product, decreased after 3 and 7 days of storage to 33.35 and 38.64 mg GAE/100 g product and increased again after 14 and 21 days of storage to 51.67 and 55.37 mg GAE/100 g product (table 5). The addition of 2.5% turmeric and black pepper essential oils to curd gave the highest total polyphenols content, which decreased significantly after 24 hours from 154.79 mg GAE/100 g product to 122.18 mg GAE/100 g product after 21 days of storage. Our previous studies on the total polyphenols gave lower results when adding dry matter in different concentrations of turmeric with black pepper - from 13.33 to 29.50 mg GAE/100 g product and 4.67 mg GAE/100 g product in the control curd [20].

Saturated fatty acids (SFA) in the control curd group were 72.67 g/100g fat and increased significantly ($P \geq 0.001$) when adding turmeric and black pepper EO in all variants, with the lowest content being found when adding 1.5% -74.99 g/100g fat on the 24 hours. Monounsaturated fatty acids decreased as a result of the supplementation of TBPEO except 1 and 2.5%, respectively 23.44 g/100g fat and 24.27 g/100g fat, while to decreased the polyunsaturated fatty acids (table 6). Trans isomers of oleic acid increased with the addition of essential oils from 4.81 g/100g fat in the control curd to 7.91 g/100g fat at 0.75% supplement. Cis isomers decreased from 12.73 g/100g fat in control between 0.5% supplement- 8.48 g/100g fat. Conjugated linoleic acid varied within narrow limits from 0.22 g/100g fat in the control group to 0.15 g/100g fat with 0.5% add- on. The content of omega-3 fatty acids in the studied curd was 0.55 g/100g fat and decreased depending on the amount of added turmeric and black pepper essential oil, with the lowest amount found at 0.5% supplement - 0.24 g/100g fat. Omega-6 fatty acids decreased from 3.11 g/100g fat in the control curd to 2.28 g/100g fat with 0.5% TBPEO. The ratio of omega-6 and omega-3 in the control group was 5.7 and due to decrease in the content of omega-6 and omega-3 after amplification of turmeric and black pepper essential oil. The best quotient between them was achieved at 1 and 1.5%, respectively 4.92 and 3.01. Branched-chain fatty acids per 24 hours in the curd decreased in all variants except for the addition of 0.75% essential oil- 3.23 g/100g fat compared to the control- 3.08 g/100g fat, which was an indicator of microbiological activity. Measure up to our previous studies, adding turmeric and black pepper by dry substances in curd consequent to higher results for the saturated fatty acids, conjugated linoleic acid, omega-3, omega-6 and branched-chain fatty acids, and lower results for poly- and monounsaturated fatty acids, trans and cis fatty acids content [20].

SFA after three days of storage in refrigerated conditions increased to 74.54 g/100g fat in control and increased significantly ($P \geq 0.001$) compared to 24 h (table 6 and 7). The treated curd with an additive gave the same changes with the exception of 0.75%, where the content was maintained - 77.15 g/100g fat and at 2% a decrease to 70.35 g/100g fat was established. Monounsaturated fatty acids increased low significantly ($P \geq 0.05$) in the control group to 23.07 g/100g fat, 0.5% - 20.62 g/100g fat and 2% - 27.01 g/100g fat, while in the other variants with TBPEO they decreased. In the case of polyunsaturated fatty acids, a decrease in their concentration was found after storage in refrigerated conditions for three days, with the exception of curd with the addition of 0.5%, 1% and 1.5%, in which they increased to 3.22; 3.25 and 3.48 g/100g fat, respectively.

Table 6. Fatty acids on the 24 h, g/100 g fat (n=6)

	K		0.5%		0.75%		1%		1.5%		2%		2.5%	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
SFA	72.67	0.99	76.09	1.31	77.97	0.04	75.31	0.12	74.99	3.49	78.56	0.07	74.12	0.03
MUFA	20.91	0.28	16.44	0.28	20.52	0.01	23.44	0.04	19.34	0.90	20.03	0.02	24.27	0.01
PUFA	3.67	0.05	2.80	0.05	2.83	0.00	2.85	0.00	2.90	0.14	3.05	0.00	2.67	0.00
∑ C-18:1Trans-FA	4.81	0.07	4.40	0.08	7.91	0.00	6.96	0.01	6.95	0.32	7.29	0.01	6.20	0.00
ΣCLA	0.72	0.01	0.58	0.01	0.33	0.00	0.33	0.00	0.24	0.01	0.65	0.00	0.31	0.00
C-16:0/C-18:1cis9	3.47	0.00	5.22	0.00	5.33	0.00	5.08	0.00	5.87	0.00	6.57	0.00	6.04	0.00
C-16:0/C-18:1 ges.	2.04	0.00	2.88	0.00	2.23	0.00	2.12	0.00	2.34	0.00	2.43	0.00	2.38	0.00
Σ n-3	0.55	0.01	0.24	0.00	0.40	0.00	0.53	0.00	0.84	0.04	0.30	0.00	0.32	0.00
Σn-6	3.11	0.04	2.28	0.04	2.50	0.00	2.60	0.00	2.52	0.12	2.81	0.00	2.87	0.00
Σ MCT(C-10>C-14)	21.15	0.29	23.91	0.41	22.75	0.01	22.77	0.04	22.37	1.04	23.60	0.02	23.87	0.01
Σ SCT(C-4>C-8)	6.63	0.09	6.36	0.11	8.08	0.00	8.19	0.01	7.89	0.37	7.83	0.01	7.48	0.00
CLA 9c.11t	0.22	0.00	0.15	0.00	0.21	0.00	0.23	0.00	0.17	0.01	0.22	0.00	0.19	0.00
Σ n-6/Σn-3	5.70	0.00	9.42	0.00	6.29	0.00	4.92	0.00	3.01	0.00	9.54	0.00	8.99	0.00
ΣC-18:1cis-FA	12.73	0.17	8.48	0.15	9.07	0.00	9.69	0.01	8.41	0.39	8.44	0.01	8.86	0.00
BFA	3.08	0.04	2.31	0.04	3.23	0.00	2.10	0.00	2.49	0.12	2.87	0.00	2.18	0.00

a- K/ 0.5%; b- K/ 0.75%; c- K/ 1%; d-K/ 1.5%; e- K/ 2%; f- K/ 2.5%; P≤0.001

Table 7. Fatty acids on the 3 day, g/100 g fat (n=6)

	K		0.5%		0.75%		1%		1.5%		2%		2.5%	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
SFA	74.54	0.05	77.93	0.09	77.15	1.53	76.58	0.03	77.77	0.10	70.35	0.88	78.80	0.29
MUFA	23.07	0.01	20.62	0.02	19.65	0.39	22.19	0.01	17.66	4.27	27.01	0.30	20.41	0.08
PUFA	3.11	0.00	3.22	0.00	2.84	0.06	3.25	0.00	3.48	0.10	2.06	0.03	2.27	0.01
∑ C-18:1Trans-FA	6.43	0.00	6.74	0.01	7.29	0.14	6.07	0.00	4.72	3.30	0.00	0.00	0.00	0.00
ΣCLA	0.44	0.00	0.22	0.00	0.41	0.01	0.37	0.00	0.29	0.00	0.29	0.00	0.27	0.00
C-16:0/C-18:1cis9	5.13	0.00	4.34	0.00	4.98	0.00	4.37	0.00	5.32	0.00	2.43	0.00	2.45	0.00
C-16:0/C-18:1 ges.	1.96	0.00	2.21	0.00	2.27	0.00	2.24	0.00	2.80	0.97	2.43	0.00	2.45	0.00
Σ n-3	0.48	0.00	0.40	0.00	0.45	0.01	0.48	0.00	0.40	0.00	0.57	0.01	0.24	0.00
Σn-6	3.35	0.00	2.99	0.00	2.39	0.05	3.00	0.00	3.55	0.10	1.20	0.02	1.76	0.01
Σ MCT(C-10>C-14)	23.81	0.02	22.86	0.03	22.34	0.44	24.17	0.01	23.11	0.13	22.81	0.24	23.55	0.09
Σ SCT(C-4>C-8)	9.25	0.01	9.53	0.01	9.42	0.19	8.56	0.00	7.90	0.01	6.85	0.09	8.80	0.03
CLA 9c,11t	0.28	0.00	0.13	0.00	0.18	0.00	0.23	0.00	0.24	0.00	0.24	0.00	0.24	0.00
Σ n-6/Σn-3	7.05	0.00	7.54	0.00	5.32	0.00	6.18	0.00	8.81	0.25	2.11	0.00	7.50	0.00
ΣC-18:1cis-FA	10.75	0.01	9.95	0.01	8.82	0.17	9.84	0.00	9.81	0.90	12.57	0.16	14.98	0.06
BFA	3.64	0.00	3.22	0.00	3.21	0.06	2.92	0.00	2.87	0.01	3.06	0.04	2.83	0.01

Table 8. Fatty acids on the 7 day, g/100 g fat (n=6)

	K		0.5%		0.75%		1%		1.5%		2%		2.5%	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
SFA	68.67	0.33	74.52	1.60	73.94	0.42	77.71	0.14	77.63	1.10	63.06	0.58	63.03	1.19
MUFA	23.71	0.11	21.64	1.42	21.94	0.12	19.68	0.04	18.97	0.27	30.44	0.28	25.82	0.49
PUFA	3.13	0.02	3.22	0.50	3.47	0.02	3.81	0.01	3.98	0.06	3.31	0.03	2.25	0.04
∑ C-18:1Trans-FA	0.00	0.00	6.02	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ΣCLA	0.44	0.00	0.26	0.02	0.16	0.00	0.66	0.00	1.36	0.02	0.08	0.00	0.01	0.00
C-16:0/C-18:1cis9	2.26	0.00	3.90	0.30	2.30	0.00	2.44	0.00	2.46	0.00	1.66	0.00	2.15	0.00
C-16:0/C-18:1 ges.	2.21	0.00	2.13	0.16	2.30	0.00	2.44	0.00	2.46	0.00	1.66	0.00	2.07	0.00
Σ n-3	0.52	0.00	0.41	0.06	0.80	0.00	0.46	0.00	0.20	0.00	0.42	0.00	0.00	0.00
Σn-6	2.18	0.01	2.94	0.46	2.51	0.01	2.69	0.00	2.42	0.03	2.81	0.03	2.24	0.04
Σ MCT(C-10>C-14)	23.45	0.11	22.22	0.25	21.72	0.12	22.64	0.04	23.45	0.33	16.50	0.15	20.89	0.39
Σ SCT(C-4>C-8)	0.42	0.00	8.72	0.42	8.70	0.05	8.58	0.02	9.13	0.13	5.25	0.05	8.84	0.17
CLA 9c,11t	0.20	0.00	0.14	0.02	0.08	0.00	0.21	0.00	0.12	0.00	0.08	0.00	0.00	0.00
Σ n-6/Σn-3	4.17	0.00	7.23	0.02	3.12	0.00	5.89	0.00	11.92	0.00	6.69	0.00	0.00	0.00
ΣC-18:1cis-FA	16.29	0.08	10.51	0.55	14.77	0.08	15.38	0.03	14.90	0.21	19.04	0.17	14.68	0.28
BFA	2.69	0.01	3.25	0.05	3.10	0.02	2.54	0.00	2.10	0.03	1.87	0.02	1.07	0.02

Table 9. Fatty acids on the 14 day, g/100 g fat (n=6)

	K		0.5%		0.75%		1%		1.5%		2%		2.5%	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
SFA	65.18	3.41	73.87	2.57	71.37	1.06	73.34	0.76	75.63	3.12	64.39	0.70	62.19	0.58
MUFA	24.84	1.30	21.38	0.74	24.24	0.36	24.02	0.27	20.60	0.85	27.96	0.34	28.30	0.26
PUFA	4.21	0.22	2.69	0.09	2.48	0.04	2.67	0.03	2.02	0.08	1.53	0.02	1.86	0.02
∑ C-18:1Trans-FA	5.75	0.30	5.03	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ΣCLA	0.33	0.02	0.27	0.01	0.51	0.01	0.20	0.00	0.07	0.00	0.08	0.00	0.19	0.00
C-16:0/C-18:1cis9	2.93	0.00	3.83	0.00	2.41	0.00	2.27	0.00	2.44	0.00	2.55	0.00	2.31	0.00
C-16:0/C-18:1 ges.	1.70	0.00	2.22	0.00	2.41	0.00	2.27	0.00	2.44	0.00	2.55	0.00	2.31	0.00
Σ n-3	0.54	0.03	0.35	0.01	0.21	0.00	0.36	0.00	0.23	0.01	0.03	0.00	0.00	0.00
Σn-6	3.81	0.20	2.41	0.08	1.75	0.03	2.10	0.02	1.73	0.07	1.42	0.02	1.53	0.01
Σ MCT(C-10>C-14)	20.89	1.09	21.84	0.76	22.55	0.33	23.10	0.21	24.11	0.99	23.72	0.29	22.82	0.21
Σ SCT(C-4>C-8)	6.67	0.35	8.52	0.30	7.97	0.12	8.59	0.09	8.96	0.37	7.40	0.07	6.82	0.06
CLA 9c,11t	0.19	0.01	0.13	0.00	0.24	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ n-6/Σn-3	7.06	0.00	6.84	0.00	8.17	0.00	5.86	0.00	7.63	0.00	50.97	0.00	0.00	0.00
ΣC-18:1cis-FA	11.82	0.62	10.61	0.37	13.16	0.20	14.11	0.16	13.90	0.57	10.25	0.13	10.69	0.10
BFA	3.06	0.16	3.37	0.12	2.53	0.04	2.60	0.03	2.56	0.11	3.46	0.04	3.16	0.03

Table 10. Fatty acids on the 21 day, g/100 g fat (n=6)

	K		0.5%		0.75%		1%		1.5%		2%		2.5%	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
SFA	70.66	0.76	72.35	1.40	72.13	0.69	71.25	1.52	77.39	0.95	75.82	1.11	62.37	1.06
MUFA	24.51	0.26	23.93	0.46	25.93	0.25	22.72	0.48	20.48	0.25	23.31	0.34	32.40	0.55
PUFA	2.56	0.03	2.16	0.04	1.57	0.02	2.35	0.05	2.33	0.03	2.47	0.09	2.54	0.04
∑ C-18:1Trans-FA	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
ΣCLA	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
C-16:0/C-18:1cis9	2.15	0.00	2.26	0.00	2.34	0.00	2.15	0.00	2.44	0.00	2.26	0.00	1.61	0.00
C-16:0/C-18:1 ges.	2.15	0.00	2.26	0.00	2.34	0.00	2.15	0.00	2.44	0.00	2.26	0.00	1.61	0.00
Σ n-3	0.59	0.01	0.17	0.00	0.27	0.00	0.24	0.01	0.14	0.00	0.56	0.11	0.00	0.00
Σn-6	1.98	0.02	1.99	0.04	1.30	0.01	2.11	0.04	2.20	0.03	1.90	0.03	2.47	0.04
Σ MCT(C-10>C-14)	25.55	0.27	23.70	0.46	24.35	0.23	22.29	0.48	24.59	0.30	23.70	0.35	23.85	0.40
Σ SCT(C-4>C-8)	8.13	0.09	6.98	0.13	8.54	0.08	6.33	0.13	8.47	0.10	8.31	0.12	6.73	0.11
CLA 9c,11t	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
Σ n-6/Σn-3	3.37	0.00	11.56	0.00	4.79	0.00	8.89	0.00	16.16	0.00	3.45	0.65	0.00	0.00
ΣC-18:1cis-FA	13.53	0.14	14.68	0.28	13.02	0.12	15.54	0.33	14.57	0.18	15.19	0.22	13.63	0.23
BFA	3.50	0.04	2.52	0.05	2.71	0.03	2.93	0.06	2.61	0.03	1.68	0.02	3.64	0.06

The content of trans fatty acids increased in the control group and curd with the 0.5%, 0.75% and 1% supplementation, while at 1.5% they decreased, and at 2 and 2.5% they were not detected.

The cis isomers higher at the expense of the trans isomers of oleic acid. After storage of the curd for three days, an expend in conjugated linoleic acid was found in all variants. Omega-3 fatty acids in the control curd decreased to 0.48 g/100g fat and when using 1%, 1.5% and 2.5% addition also reduced to 0.48; 0.40 and 0.24 g/100g fat. Omega-6 fatty acids augment after three days of storage in the control curd, 0.5%, 1%, and 1.5%, while at 0.75%, 2 and 2.5% their dropped was found. The resulting changes in the content of omega-3 and omega-6 fatty acids increased the ratio of omega-6 and omega-3 fatty acids, with the exception of curds with the addition of 2% turmeric essential oil and black pepper- 2.11. Branched-chain fatty acids raised in all variants of the curd, which was a sign of microbiological activity, as well as the surge in conjugated linoleic acid.

Refrigerated storage of curd for 7 days reduces the content of saturated fatty acids at the expense of unsaturated fatty acids. Trans isomers of oleic acid were detected at 0.5%, cis isomers raised in all variants except for curd with 2.5% TBPEO, where they remain at 14.68 g/100g fat. The amount of conjugated linoleic acid decreases twofold and was not detected at 2.5% addition in curd (table 7 and 8). The content of omega-3 and omega-6 fatty acids decreases, and in curd with 2.5% supplementation, omega-3 fatty acids are not detected. After 7 days of storage, the ratio between omega-6 and omega-3 fatty acids in the control group of curd lowered to 4.17 and at 0.75% it was below 5-3.12, at 2.5% it was not recorded due to the absence of omega-3 fatty acids and in the other variants it exceeded the favourable health index. Branched-chain fatty acids reduced in all variants of the curd, with the exception of the application of 0.5% TBPEO where they were 3.25, i.e. they remained quantitatively as on the 3rd day.

As the storage of the curd progresses under refrigerated conditions, a significant ($P \geq 0.001$) dropped in saturated fatty acids was found in the control group and in the curd with different concentration of additive, but with 2% TBPEO, it increased slightly to 64.39 g/100g fat (tables 8 and 9). Trans isomers of oleic acid were detected in the curd and at 0.5% addition, cis isomers decreased in all variants compared to the seventh day, but at 0.5% (10.61 g/100g fat); 2% (10.25 g/100g fat) and 2.5% (10.69 g/100g fat) had a lower content than the control group - 11.82 g/100g fat, with the exception of the 2.5% supplementation, where they remained - 14.68 g/100g fat. The amount of conjugated linoleic acid with the exception of curds with the enhance of 2% turmeric essential oil and black pepper- 2.11. Branched-chain fatty acids increased in all variants of the curd, which was a sign of microbiological activity, as well as the increase in conjugated linoleic acid.

After 21 days shelf life of the curd in refrigerated conditions, an intensify in the content of saturated fatty acids was found in the control group - 70.66 g/100g fat and with 1 and 2% addition - 77.39 g/100g fat and 75.82 g/100g fat compared to the curd on day 14. The remaining variants with different concentrations of the supplementation of turmeric and black pepper essential oil have a diminished amount of saturated fatty acids at the expense of a sour in monounsaturated fatty acids. The content of polyunsaturated fatty acids smaller twofold compared to day 14 in the control group curd (table 9 and 10), while when using the add-on, it varied within narrow limits with a tendency to drop. Trans fatty acids and conjugated linoleic acid and its isomers after 21 days storage of the curd - control group and with the TBPEO were not found. The content of cis isomers of oleic acid increases after 21 days shelf life compared to 14 days and the raise value was recorded at 1 and 2% supplementation - 15.54 and 15.19 g/100g fat compared to the control group - 13.53 g/100g fat.

After 21 days of refrigerated storage, the amount of omega-3 and omega-6 fatty acids decreases compared to the results obtained for 14 days of shelf life and again no presence of omega-3 was recorded in curd with the 2.5% TBPEO. The ratio between the two groups of fatty acids reduced as a result of the established changes in both groups. Branched chain fatty acids higher in the control group to 3.50 g/100g fat and in the 2.5% addition - 3.64 g/100g fat, while in the other variants they were lower and diminished compared to the curd at 14 days of storage.

Conclusions

The total fat content was highest when adding 2 and 2.5% turmeric and black pepper essential oil at 24 h compared to the control curd ($P \leq 0.001$). The protein varied insignificantly both at 24 h and when monitoring its change during refrigerated storage up to 21 days. The application of TBPEO at different concentrations improved the antioxidant capacity and total polyphenol content, both depending on the amount and during the shelf life. The use of the supplement led to an escalate in saturated fatty acids at 24 h and on day 3 at the expense to reduce an unsaturated fatty acid. On the 7th and 14th days saturated fatty acids diminished and expanded again on 21st day. After 21 days of refrigerated storage, a lack of CLA and a reduce in omega-3 and omega-6 fatty acids were found in all variants.

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