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Biochemical and Microbiological Properties of Edam Cheese with Black Cumin Oil

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EDAM cheese samples were prepared with the addition of different concentrations of black cumin (*Nigella sativa* L) oil (0.2, 0.4 and 0.6 % v/w). Significant differences ($P \leq 0.05$) in chemical composition and ripening indices among treated cheese sample (0.6 % oil) and control was observed in all examined parameters. The added black cumin oil increased cheese acidity from 0.79 % in control to 1.13 % in cheese with 0.6 % oil at fresh time, with continuously increase in all cheese samples during ripening. Soluble nitrogen/ Total Nitrogen reached 15.91 % in cheese with higher level of black cumin oil at the end of ripening. Free amino acids recorded1.21 g leucine/g cheese in Edam cheese with 0.6 % oil at end of ripening times. Free fatty acids increased with increasing level of oil in Edam cheese samples. Incorporation of black cumin oil in Edam cheese reduced the total viable count (5.97 log cfu/g), yeast & molds (1.00 log cfu/g)at the end of ripening and inhibited the growth of coliform groups. Proteolytic bacteria recorded higher counts (3.19 log cfu/g), while lipolytic bacteria recorded lower counts (2.59 log cfu/g) in Edam cheese with 0.6 % oil comparing to other cheese samples at 60 days of ripening. Panelists accepted the taste of Edam cheese with higher concentration of black cumin oil (0.6 %) with no complains on appearance and smell, while they favored the texture of Edam cheese with higher percentage of oil, then overall acceptability went to 0.6 % oil treated cheese.

Keywords: Edam cheese, Black cumin oil, Ripening indices, Microbiological properties.

Introduction

Edam cheese, a semi hard cheese variety, is one of the main types of cheese manufactured in Netherlands, which is manufactured in the form of sphere loaf weighing about 0.2 - 20 kg, which contain about 40 – 44 % fat content in solid matter (F/DM) and ripened for two weeks to around two years. The quality characteristics of the produced cheese are determined mainly by the quality of cheese milk which must have good bacteriological quality and a standardized chemical composition (Fox et al., 2017). The spoilage and pathogenicmicroorganisms which can grow in cheese loaf are the major problems facing Edam cheese industry and cause some defects which may affect the quality and shelf life of resultant cheese (Doyle, 2009). It is common to use nitrates (NO_3), which is converted inside the cheese matrix into nitrite (NO_2) to avoid early blowing by coliforms and preventing the growth of clostridia germs, but it is often undesirable and not allowed to add nitrate to the cheese milk (Van den Berg et al., 2004). Great consumer awareness and concern regarding synthetic chemical additives have led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity (Marino et al., 2001). Plant essential oils are gaining interest for their potentials as preservative ingredients or decontaminating treatments as they have GRAS stats and a wide acceptance from consumers (Burt,2004). Black cumin (*Nigella sativa* L) oil is one of the natural antibacterial and antifungal, which prevent the growth of numerous pathogenic and defective bacteria present in food matrices. It is known also for its valuable content of phenolic compounds, flavonoids, phytosterols, fatty acids, vitamins, minerals and some volatile compounds (Çakır and Çakmakçı, 2018). The oil extract of *Nigella*

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sativa showed in vitro and in vivo antimicrobial effect against broad spectrum of pathogenic bacteria (Mashhadian and Rakhshandeh, 2005). It has been used in many food applications as antibacterial and antifungal and also due to its health benefits (Tarakciet al., 2005; Cakir et al., 2016 and Georgescu et al., 2018). Therefore, the objective of this study was to evaluate the effect of using black cumin oil on the microbiological and quality characteristics of Edam cheese during ripening.

Materials and Methods

Fresh cow's milk was obtained from the herd of animal production, Faculty of Agriculture, Fayoum University, Fayoum Governorate, Egypt. Black cumin oil (*Nigella sativa* L) was supplied by ALREHAB HERBS Company, Fayoum, Egypt.Edam cheese starter culture (FD-DVS CHN-11, mesophilic aromatic culture consist of *Lactococcus lactis subsp. cremoris*, *Leuconostoc, Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis biovar diacetylactis*) was supplied by MIFAD company for food additives, Cairo, Egypt.Rennet powder (CHY-MAX, 2280 IMCU/ml) was obtained from Ch. Hansen Lab., Denmark. Commercial pure fine grade salt (NaCl) was obtained from Fayoum Governorate, Egypt, [Emisal Company]. Calcium chloride (Food quality grade) was obtained from EL-Nasr Company, Cairo, Egypt. Chemicals used in this study were analytical grade and obtained from El-Nasser, Merckand Sigma Companies.

Analysis of black cumin oil

Chromatographic analysis was used to determine the Fatty acids and sterol composition of black cumin oilaccording to the method used by Ramadan et al. (2010), while the total phenolic compounds were determined according to the method used by Hassanien et al. (2014).

Procedure of Edam cheese making

Edam cheese was manufactured according to Sabikhi et al. (2014) with some modifications as shown in Fig. 1.

Chemical analysis of Edam cheese

Titratable acidity, fat, moisture, ash, total nitrogen (TN) and water soluble nitrogen (WSN) contents were determined as described in AOAC (2019).The pH values of samples were measured using laboratory pH meter with a glass electrode Model pH-(Kent EIL 7020). Moisture in fat-free base (MFFB) was calculated using the total solids and fat contents with the equation:

MFFB = * 100

Chromatographic analysis of Edam cheese samples was performed to determine the fatty acids composition which is done according to Ramadan et al. (2010). Analysis of the free amino acids (FAA) was done according to the method of Folkertsma (1992).

Microbiological analysis

Bacterial count of different groups (total viable counts (TVC), yeast & mold counts, Coliform, proteolytic and lipolytic bacteria) were assayed according to APHA (2004), (Oxoid Limited, Basingstoke, UK).

Sensory evaluation

Sensory evaluation was determined using a nine-point hedonic scale as the method used by Amini et al. (2019). The samples of cheese were evaluated by 15 semi trained panelists for appearance, taste, texture, smell, and overall acceptability.

Statistical analysis

Data were statistically analyzed using ANOVA variance analysis through the general linear model (GLM) procedure of the statistical analysis system software (SAS version 9.1, SAS Institute, Inc., (SAS, 2003). The model included treatments, ripening periods, and their interaction as fixed effects. Differences between effects were assessed by the Duncan test ($P \leq 0.05$).

Results and Discussion

Fatty acids and sterol composition of black cumin oil (BCO)

The level of saturated and unsaturated fatty acids of BCO was 15.67 % and 84.33, respectively (Table 1). GC-MS analysis of BCO detected the main fatty acids present which dominated by the essential omega-6 fatty acid, linoleic acid (C18:2) 55.69 % followed by oleic acid (C18:1)27.92 %, palmitic acid (C16:0) 12.19 %, stearic acid (C18:0) 3.19 %, linolenic acid (C18:3) 0.53 %, palmitoleic acid (C16:1) 0.19 %, myristic acid (C14:0) 0.17 % and arachidic acid (C20:0) 0.12 %. In addition, seven phytosterol compounds were also present in BCO (Table 1) with the major ß-sitosterol 48.7 % followed by stigmasterol 16.9 %, campesterol 12.6 %, Δ5-avenasterol 12.1 %, Δ7-avenasterol 2 %, cholesterol 0.8 % and Δ7- stigmasterol 0.7 %. Results in Table 1 also revealed that BCO had a high level of phenolics 3.8 g/kg.

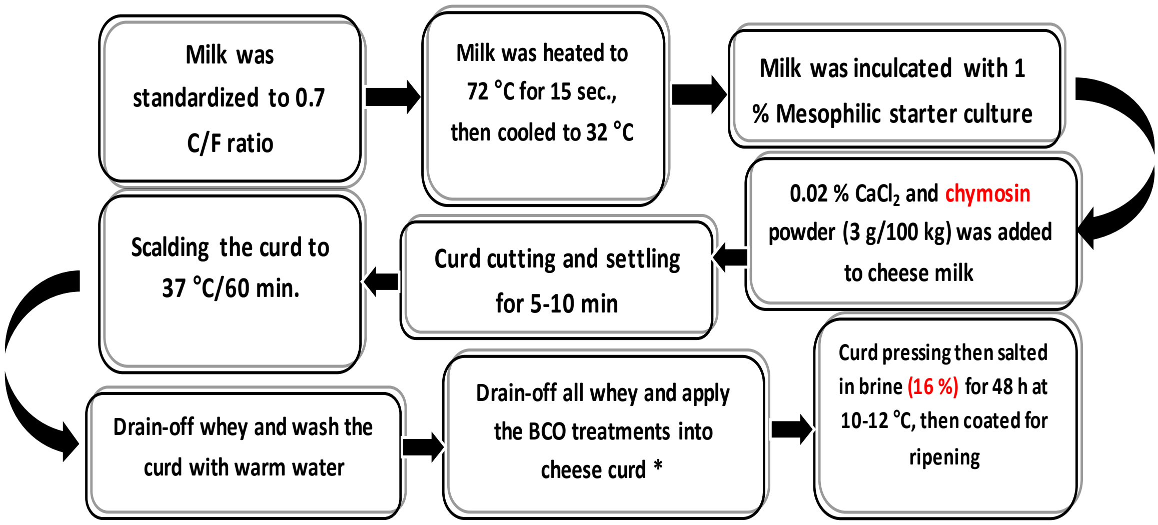

Fig. 1. Procedure for Edam cheese making
*** BCO treatments (0, 0.2, 0.4 and 0.6 % v/w)**

TABLE 1. Fatty acids and phytosterols percentage, total phenolics of black cumin oil

Component	Percentage
Fatty acids	
Myristic acid (C14:0)	0.17
Palmitic acid (C16:0)	12.19
Palmitoleic acid (C16:1)	0.19
Stearic acid (C18:0)	3.19
Oleic acid (C18:1)	27.92
Linoleic acid (C18:2)	55.69
Linolenic acid (C18:3)	0.53
Arachidic acid (C20:0)	0.12
Σ Saturated fatty acids	15.67
Σ Unsaturated fatty acids	84.33
Phytosterols	
Cholesterol	0.8
Campesterol	12.6
Stigmasterol	16.9
ß-Sitosterol	48.7
Δ5-Avenasterol	12.1
Δ7-Stigmasterol	0.7
Δ7-Avenasterol	2.00
Total phenolics	3.8 g/kg

BCO have been used in numerous food applications due to its high content of phenolic compounds with antioxidant activities and also due to it valuable content of unsaturated fatty acids and phytosterols. It was reported that the fatty acids of BCO play an important role in the biological activities and might be a health benefit ingredient when added to food material beside its promoting effect for prolonging shelf life of foods (Hassanien et al., 2015).

Chemical composition of Edam cheese

The effect of added black cumin oil on the chemical composition of Edam cheese is presented in Table 2. The moisture contents of Edam cheese with added BCO were higher than the control Edam cheese, and significant differences ($P \leq 0.05$) were observed between treated Edam cheese with 0.6 % BCO and control cheese. The highest moisture content 45.91 % was obtained in Edam cheese with higher concentration (0.6 %) of BCO at fresh time, while the lowest moisture content 45.04 % was obtained in control cheese. There was a gradual decrease in moisture content in all cheese samples during ripening except the first fifteen days were the moisture decreased rapidly. BCO treated Edam cheese recorded higher moisture contents when compared to control cheese at the end of ripening period, with higher moisture retention in Edam cheese with higher BCO concentration. The moisture values were 36.07, 37.82, 38.33 and 39.47 % in control, T1, T2 and T3, respectively at the end of ripening period.The decrease in moisture content could be due to the evaporation of water from cheese during ripening and the effect of microbial growth and the development of cheese acidity (Çakır and Çakmakçı (2018). These results were in agreement with Hassanien et al. (2014) who found that soft cheese supplemented with black cumin oil had higher moisture contents when compared to control cheese.

The titratable acidity increased throughout the ripening of all Edam cheese samples with statistically significant differences (P≤0.05) between the cheese samples with different oil concentrations. The highest acidity 1.13 % was observed in Edam cheese with 0.6 % BCO, while the lowest acidity 0.79 % was recorded in control Edam cheese at fresh time. This could be explained by the acidity effect of BCO due to its content of fatty acids and acidic components (Çakır and Çakmakçı, 2018). Edam cheese samples recorded 1.26, 1.37, 1.49 and 1.59 % acidity for control, T1, T2 and T3, respectively at the end of ripening period.

In addition, the fermentation of the residual lactose by cheese flora may confer the values of cheese acidity. These findings were in line with (Hamid, 2014) who used cumin oil in the manufacture of Sudanese white cheese. Also, these findings were in line with El-Aidie et al. (2019) who revealed that cheese acidity increased gradually during the two months of Edam cheese ripening which was due to the lactose fermentation by lactic acid bacteria. Vice versa, pH values (Fig. 2) decreased in all Edam cheese samples during ripening period with higher decrease in cheese samples with higher BCO concentration. These results were in agreement with Abdel-Razig et al. (2014) and El-Aidie et al. (2019). The formation of volatile acids throughout ripening of cheese might interfere with recorded cheese acidity and pH values. The development of acidity and lowering pH values may also be due to the fact that BCO does not affect the growth of lactic acid bacteria responsible for acidity production in the cheese sample (Georgescu et al., 2018).

Although, the increase of BCO concentration between the different treatments, the results revealed that BCO had a non-significant effect (p≤0.05) on the fat percentage of all Edam cheese samples. The highest fat percentage 24.67 % was determined in Edam cheese with 0.6 % BCO while the lowest fat percentage 23.83 % was determined in control Edam cheese. Due to the increasing dry matter content of cheese samples throughout ripening period, the fat content of all Edam cheese samples increased gradually till the end of ripening period (Hassanien et al., 2014).Likewise, the protein content of all Edam cheese samples increased during ripening period with non-significant differences (P≤0.05) between cheese samples with different concentrations of BCO as reported by Hamid (2014) who used cumin oil in Sudanese white cheese and reported the increase in protein content of treated cheese when compared to control cheese. The F/DM contents of Edam cheese increased during ripening with statistically non-significant (P≤0.05) differences among cheese samples. Although, F/DM contents conformed to the Egyptian standards which indicated the F/DM contents to be in range 40 % to over 50 %. The changes in F/DM contents might be due to the variations in moisture and fat contents of Edam cheese during ripening period (ES: 1007-3/2005).

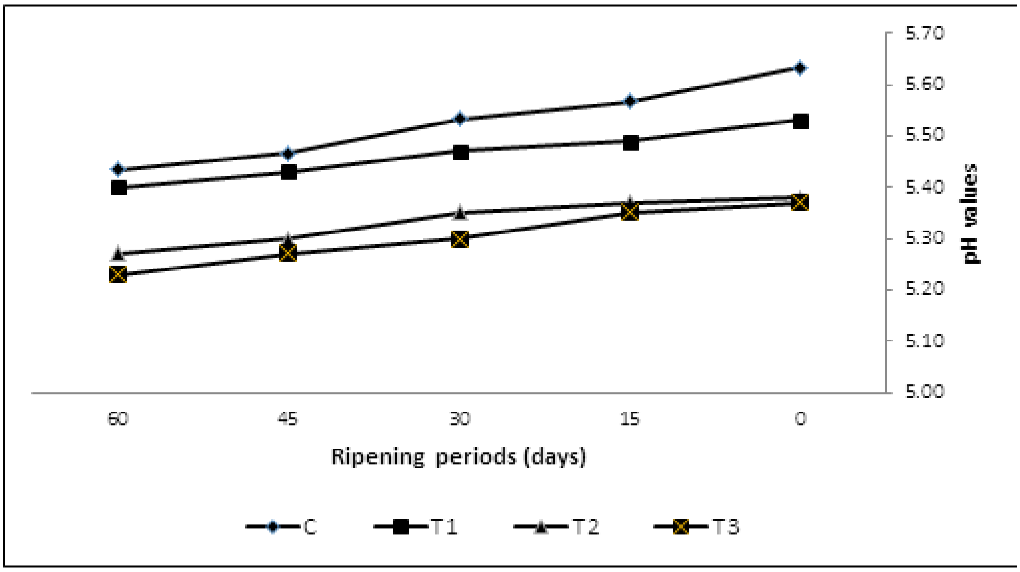

Fig. 2. pH values of Edam cheese samples with different BCO concentrations during ripening periods.

TABLE 2. Effect of BCO concentrations on some chemical properties of Edam cheese during ripening periods

Parameters	Ripening periods (days)	Treatments			
		Control	T1 (0.2 % BCO)	T2 (0.4 % BCO)	T3 (0.6 % BCO)
Acidity%	0	0.790.03±$_{l}$	0.900.01±$_{k}$	1.030.06±$_{j}$	1.130.07±$_{i}$
	15	0.940.02±$_{k}$	1.020.07±$_{j}$	1.180.02±$_{hi}$	1.240.02±$_{fg}$
	30	1.140.02±$_{i}$	1.240.02±$_{fg}$	1.340.04±$_{e}$	1.420.03±$_{cd}$
	45	1.200.01±$_{gh}$	1.320.03±$_{e}$	1.430.02±$_{c}$	1.510.02±$_{b}$
	60	1.260.01±$_{f}$	1.370.02±$_{de}$	1.490.01±$_{b}$	1.590.01±$_{a}$
Moisture%	0	45.04±0.31$_{c}$	45.39±0.15$_{bc}$	45.70±0.21$_{ab}$	45.91±0.32$_{a}$
	15	41.79±0.10$_{f}$	42.71±0.70$_{e}$	43.97±0.16$_{d}$	44.06±0.31$_{d}$
	30	40.34±0.50$_{i}$	40.99±0.19$_{gh}$	41.06±0.12$_{gh}$	42.77±0.18$_{e}$
	45	38.39±0.13$_{k}$	40.50±0.34$_{i}$	40.65±0.29$_{hi}$	41.18±0.24$_{g}$
	60	36.07±0.19$_{m}$	37.82±0.32$_{l}$	38.33±0.24$_{k}$	39.47±0.20$_{j}$
Fat %	0	23.830.76±$_{i}$	24.000.00±$_{i}$	24.170.29±$_{hi}$	24.670.29±$_{h}$
	15	25.500.50±$_{g}$	26.830.29±$_{f}$	27.000.00±$_{ef}$	27.170.29±d$_{ef}$
	30	26.830.29±$_{f}$	27.000.00±$_{ef}$	27.170.29±$_{def}$	27.500.00±$_{cde}$
	45	27.670.29±$_{cd}$	28.000.50±$_{c}$	28.670.29±$_{b}$	29.170.29±$_{b}$
	60	29.000.00±$_{b}$	29.830.29±$_{a}$	30.000.00±$_{a}$	30.330.29±$_{a}$
Fat/dry matter %	0	43.371.28±$_{k}$	43.950.12±$_{jk}$	44.500.37±$_{ij}$	45.600.33±$_{gh}$
	15	43.810.91±$_{jk}$	46.850.87±$_{ef}$	48.190.13±$_{c}$	48.560.43±$_{c}$
	30	44.980.50±$_{hi}$	45.760.14±$_{gh}$	46.090.48±$_{fg}$	48.060.15±$_{c}$
	45	44.910.40±$_{hi}$	47.061.11±$_{de}$	48.300.64±$_{c}$	49.590.36±$_{ab}$
	60	45.360.14±$_{ghi}$	47.980.64±$_{cd}$	48.640.19±$_{bc}$	50.120.34±$_{a}$
Protein%	0	20.530.12±$_{j}$	20.620.06±$_{j}$	20.750.10±$_{i}$	20.810.14±$_{i}$
	15	22.590.03±$_{h}$	22.620.02±$_{h}$	22.800.02±$_{g}$	22.880.11±$_{g}$
	30	23.610.02±$_{f}$	23.680.08±e$_{f}$	23.790.01±$_{de}$	23.850.04±$_{d}$
	45	24.010.10±$_{c}$	24.190.02±$_{b}$	24.200.02±$_{b}$	24.240.02±$_{b}$
	60	24.570.08±$_{a}$	24.590.01±$_{a}$	24.490.02±$_{a}$	24.600.01±$_{a}$
MFFB %	0	59.140.51±$_{c}$	59.720.19±$_{bc}$	60.260.06±$_{ab}$	60.940.26±$_{a}$
	15	56.090.47±$_{g}$	58.381.05±$_{de}$	60.240.21±$_{ab}$	60.490.36±$_{a}$
	30	55.130.65±$_{h}$	56.150.26±$_{g}$	56.380.25±$_{fg}$	59.000.25±$_{cd}$
	45	53.070.12±$_{j}$	56.250.86±$_{fg}$	56.990.57±$_{f}$	58.140.22±$_{e}$
	60	50.800.27±$_{k}$	53.900.61±$_{i}$	54.750.35±$_{h}$	56.660.14±$_{fg}$
Ash %	0	2.920.15±$_{g}$	3.100.52±$_{efg}$	3.330.39±$_{bcdef}$	3.410.34±$_{abcd}$
	15	3.070.08±$_{fg}$	3.270.15±$_{cdef}$	3.400.11±$_{abcde}$	3.490.06±$_{abc}$
	30	3.130.09±$_{defg}$	3.470.10±$_{abc}$	3.490.09±$_{abc}$	3.510.12±$_{abc}$
	45	3.240.09±$_{cdef}$	3.500.17±$_{abc}$	3.510.02±$_{abc}$	3.640.05±$_{ab}$
	60	3.260.02±$_{cdef}$	3.520.01±$_{abc}$	3.610.08±$_{ab}$	3.690.01±$_{a}$

[a, b, c] Means ± SD in the different letters followed by different column are significantly different (P≤0.05)

MFFB= moisture in fat-free base.

Also, as shown in Table 2, the ash contents of Edam cheese samples differed non-significantly (P≤0.05) among cheese samples with different concentrations of BCO. The higher the BCO concentration, the higher the ash content of cheese sample. Throughout ripening, the ash content increased in all cheese samples due to the elevation of total solids upon water loss from cheese loafs.These results may be also due to the mineral content of BCO and it was in line with (Hamid, 2014) in Sudanese white soft cheese supplemented with cumin oil.

Ripening indices

The ripening indices of different Edam cheese treatments are shown in Table 3. Edam cheese with BCO had higher ripening indices when compared to control cheese. Hence, as the BCO concentration increased, the ripening indices increased in treated Edam cheese.The SN/TN and FAA increased gradually in all Edam cheese throughout ripening period with statistically significant differences (P≤0.05) between treated Edam cheese and control samples, with statistically non-significant differences (P≤0.05) among Edam cheese with three BCO concentrations. The higher SN/TN and free amino acids (FAA) contents were recorded with Edam cheese with higher concentration of BCO, while the lower SN/TN and FAA contents were present in control cheese.These might be explained by the effect of BCO on the proteolysis pattern of Edam cheese by enhancing the microorganisms and their enzyme activities on protein breakdown and formation of soluble nitrogenous compounds and free amino acids. Moreover, the enhanced growth of proteolytic bacteria in Edam cheese which characterizes with higher moisture contents. These findings were in agreement with Jasinska et al. (2007).

The different proteolytic pattern of Edam cheese samples might be also due to the variations in protein contents among different Edam cheese samples. On the other hand, the higher moisture in fat-free base (MFFB) in Edam cheese (Table 2) with BCO might enhance the proteolytic pathway.

The fatty acid composition of different Edam cheese samples are shown in Table 4. Higher levels of saturated fatty acids were detected in Edam cheese with BCO, 10.73, 10.76 and 10.79 g/100g cheese for T1, T2 and T3, respectively comparing to 10.7 g/100g in control cheese. In addition, BCO treated samples had higher levels of unsaturated fatty acids 7.41, 7.58 and 7.75 g/100g cheese for T1, T2 and T3 respectively comparing to 7.24 g/100g in control cheese. It is obvious that the higher

TABLE 3. Effect of BCO concentrations on ripening indices of Edam cheese during ripening

Parameters	Ripening periods (days)	Treatments			
		Control	T1 (0.2 % BCO)	T2 (0.4 % BCO)	T3 (0.6 % BCO)
SN/TN %	0	9.320.36±$_{l}$	10.010.49±$_{k}$	10.660.45±$_{ij}$	11.240.25±$_{gh}$
	15	9.410.42±$_{l}$	11.280.29±$_{g}$	13.150.28±$_{f}$	13.940.22±$_{de}$
	30	10.180.41±$_{jk}$	13.200.31±$_{f}$	13.680.27±$_{ef}$	14.800.42±$_{c}$
	45	10.720.12±$_{hij}$	14.240.71±$_{d}$	14.850.40±$_{c}$	15.090.41±$_{c}$
	60	10.990.43±$_{ghi}$	15.310.26±$_{bc}$	15.740.38±$_{ab}$	15.910.14±$_{a}$
FAA mg leucine/g	0	0.660.04±$_{i}$	0.720.03±$_{hi}$	0.850.03±f$_{g}$	0.870.15±$_{ef}$
	15	0.710.01±$_{i}$	0.810.01±$_{fg}$	0.930.03±d$_{e}$	0.960.01±$_{cd}$
	30	0.790.01±$_{gh}$	0.870.02±$_{ef}$	0.980.02±$_{cd}$	0.990.01±$_{cd}$
	45	0.810.01±$_{fg}$	0.950.04±$_{d}$	1.030.06±$_{bc}$	1.060.06±$_{b}$
	60	0.850.01±$_{fg}$	1.150.03±$_{a}$	1.190.01±$_{a}$	1.210.02±$_{a}$

[a, b, c] Means ± SD in the different letters followed by different column are significantly different (P≤0.05)

FAA: free amino acids SN: soluble nitrogen TN: total nitrogen level of unsaturated fatty acid in BCO affected the fatty acid content in Edam cheese samples. Fatty acid profile of Edam cheese samples with different levels of BCO was dominated by oleic acid (C18:1) 6.17, 6.22 and 6.28 g/100g in T1, T2 and T3 respectively in comparison to 6.11 g/100 g in control cheese. Followed by palmitic acid (C16:0) 5.64, 5.67 and 5.69 g/100 g for T1, T2 and T3, respectively in comparison to 5.62 g/100 g in control cheese. This could be explained by the different fat contents in Edam cheese samples. Also, the acidity of cheese and its moisture content may affect the level of fatty acids.These results were in line with Hassanien et al. (2014) who revealed that higher concentrations of BCO with its higher content of fatty acids may encourage lactic acid bacteria for hydrolyzing these free fatty acids and producing the flavoring acetaldehyde and di-acetyland reveal cheese flavor. Myristic acid (C14:0), palmitoleic acid (C16:1) and linolenic acid (C18:3) was in the same level in all cheese samples 2.41 g/100g, 0.52 g/100g and 0.32 g/100g cheese respectively.Linoleic acid (C18:2) was in higher levels 0.40, 0.51 and 0.62 g/100 g for BCO treated samples, T1, T2 and T3 respectively. This could be due to the higher level of linoleic acid in BCO.

Microbiological analysis

Data for the microbiological analysis are presented in Table 5. It was found that the total viable count TVC in all Edam cheese samples decreased gradually throughout ripening period. Statistically significant decrease ($P \leq 0.05$) was observed between Edam cheese samples particularly at the last month of ripening. The higher number of TVC 6.48 log cfu/g was recorded with the control cheese, while the lower number of TVC 5.97 log cfu/g was present in Edam cheese with higher concentration of BCO and there was a higher reduction of TVC in Edam cheese with BCO.The apparent decrease of TVC might be due to the effect of salt diffusion in cheese matrix and increasing salt concentration due to the loss of water, in addition to the marked increase in acidity during the ripening period and the antibacterial effect of BCO due to its content of phenolics and fatty acidy. Moreover, the bacterial autolysis could affect the number of TVC in ripened cheese. These results were in agreement with Çakır and Çakmakçı (2018) for Tulum cheese with added black cumin.

It was found by Badawi et al. (2009) that the use of BCO in the manufacture of soft white cheese inhibited the growth of coliform bacteria and to some extent the growth of lipolytic and proteolytic bacteria when used at 0.5 % and decreased the total bacterial counts.Coliformbacteria were not detected in Edam cheese containing BCO which confirm the antimicrobial effect of BCO against this type of microorganisms. These results were in accordance with Saláková et al. (2019) who applied using some essential oils as natural antimicrobial in the packaging films to inhibit the bacterial growth and prolong the shelf life of Edam cheese packed under foil. The inhibition and antibacterial effect of BCO was due to the existence of Thymoqinone TQ (2-isopropyl-5-methyl-benzoquinone) the main component of black cumin volatile oil, which inhibits DNA, RNA and protein synthesis of bacterial cell (Kahsai,2003). In addition, α-Pinene (The unsaturated bicyclic monoterpene hydrocarbon) which also present in BCO has an antibacterial activity (Ani et al., 2006).

TABLE 4. Fatty acid profile of Edam cheese with different BCO concentrations

Fatty acids	C (g/100g)	T1 0.2 % BCO (g/100g)	T2 0.4 % BCO (g/100g)	T3 0.6 % BCO (g/100g)
Myristic acid (C14:0)	2.41	2.41	2.41	2.41
Palmitic acid (C16:0)	5.62	5.64	5.67	5.69
Palmitoleic acid (C16:1)	0.52	0.52	0.52	0.52
Stearic acid (C18:0)	2.36	2.37	2.37	2.38
Oleic acid (C18:1)	6.11	6.17	6.22	6.28
Linoleic acid (C18:2)	0.29	0.40	0.51	0.62
Linolenic acid (C18:3)	0.32	0.32	0.32	0.32
Arachidic acid (C20:0)	0.31	0.31	0.31	0.31
Σ Saturated fatty acids	10.7	10.73	10.76	10.79
Σ Unsaturated fatty acids	7.24	7.41	7.58	7.75

TABLE 5. Microbiological analysis of Edam cheese with different BCO concentrations during ripening periods

Parameters	Ripening periods (days)	Treatments			
		Control	T1 (0.2 % BCO)	T2 (0.4 % BCO)	T3 (0.6 % BCO)
TVC Log cfu/g	0	6.750.01±$_{a}$	6.750.03±$_{a}$	6.740.01±$_{a}$	6.730.02±$_{a}$
	15	6.750.02±$_{a}$	6.560.00±$_{c}$	6.560.00±$_{c}$	6.540.00±$_{c}$
	30	6.630.00±$_{b}$	6.350.03±$_{e}$	6.320.04±$_{e}$	6.260.02±$_{fg}$
	45	6.530.02±$_{c}$	6.270.02±$_{f}$	6.230.02±$_{g}$	6.160.02±$_{h}$
	60	6.480.01±$_{d}$	6.190.02±$_{h}$	6.090.04±$_{i}$	5.970.02±$_{j}$
Yeast & molds Log cfu/g	0	ND	ND	ND	ND
	15	ND	ND	ND	ND
	30	1.420.10±$_{b}$	1.100.17±$_{c}$	ND	ND
	45	1.750.05±$_{a}$	1.360.10±$_{b}$	1.100.17±$_{c}$	0.330.58±$_{d}$
	60	1.970.03±$_{a}$	1.770.07±$_{a}$	1.200.17±$_{bc}$	1.000.00±$_{c}$
Coliform groups Log cfu/g	0	0.67±0.58$_{a}$	ND	ND	ND
	15	ND	ND	ND	ND
	30	ND	ND	ND	ND
	45	ND	ND	ND	ND
	60	ND	ND	ND	ND
Lipolytic bacteria Log cfu/g	0	2.360.10±$_{ghij}$	2.520.07±$_{defg}$	2.260.24±$_{ij}$	2.200.17±$_{j}$
	15	2.630.06±$_{cd}$	2.420.10±f$_{ghi}$	2.360.10±$_{ghij}$	2.300.00±$_{hij}$
	30	2.750.05±$_{abc}$	2.520.07±$_{defg}$	2.460.15±$_{efgh}$	2.420.10±$_{fghi}$
	45	2.800.04±$_{ab}$	2.590.11±$_{cde}$	2.560.07±$_{def}$	2.520.07±d$_{efg}$
	60	2.880.03±$_{a}$	2.670.06±$_{bcd}$	2.630.06±$_{cd}$	2.590.11±$_{cde}$
Proteolytic bacteria Log cfu/g	0	2.630.06±$_{k}$	2.690.09±$_{jk}$	2.690.09±$_{jk}$	2.730.05±$_{ij}$
	15	2.780.00±$_{hi}$	2.800.04±$_{ghi}$	2.820.04±$_{gh}$	2.860.03±$_{fg}$
	30	2.920.03±$_{ef}$	2.940.03±$_{ef}$	2.940.03±$_{ef}$	2.950.05±$_{e}$
	45	3.000.04±$_{de}$	3.050.08±$_{cd}$	3.130.05±$_{ab}$	3.180.03±$_{a}$
	60	3.070.04±$_{bcd}$	3.120.04±$_{abc}$	3.150.03±$_{a}$	3.190.02±$_{a}$

$^{a, b, c}$ Means ± SD in the same letters followed by different column are significantly different (P<0.05)
TVC : total viable counts cfu: colony forming unit ND: not detected

The number of yeast & molds of Edam cheese is presented in Table 5. There was no existence of yeast & molds at the first period of ripening in all Edam cheese samples. During the progress of ripening, the yeast & mold appeared in lower numbers with the highest numbers present in control cheese, while the lowest numbers accompanied with treated Edam cheeses. BCO had a significant effect on controlling the presence of yeast & molds. These findings were in line with Tarakci et al. (2005) in Tulum cheese, who found that black cumin decreased the number of yeast & molds in cheese. The decrease in yeast & molds was statistically significant (P≤0.05) in Edam cheese with higher concentration of BCO.

The numbers of proteolytic and lipolytic bacteria in Edam cheese treatments which made by different concentrations of BCO are shown in Table 5. The proteolytic and lipolytic bacteria in cheese treatments made with BCO registered the highest numbers comparing with controlEdam cheese, with the higher number in Edam cheese containing 0.6 % BCO followed by other BCO levels. Edam cheese samples incorporated with BCO at concentration of 0.2, 0.4 and 0.6 % recorded 3.12, 3.15 and 3.19 log cfu/g for proteolytic bacteria, respectively, while the same previous treatments recorded 2.67, 2.63 and 2.59 (log cfu/g) for lipolytic bacteria, respectively at the end of ripening period. There was a clear slight effect on the growth of lipolytic bacteria by black cumin oil which was obvious in the results of lipolytic bacterial counts. In overall, Edam cheese with 0.2 % BCO had the highest number of lipolytic and lowest number of proteolytic bacteria compared to other BCO cheeses, while Edam cheese with 0.6 % BCO had lowest lipolytic and highest proteolytic counts. These results were in accordance with Hamdy et al. (2017) on Ras cheese who reported the increase of lipolytic and proteolytic bacterial counts in Ras cheese during ripening and Badawi et al. (2009) who proved that black cumin oil had higher inhibition effect on the lipolytic bacterial counts than its effect on proteolytic bacterial counts.

Sensory evaluation

The clear preference of Edam cheese with BCO was related to the rate of proteolysis and the formation of aldehydes, ketones and other compounds (Cakir et al., 2016) which attract the consumer acceptability. Figure 3 show the results of a nine point hedonic scale which reveal that BCO improved the texture, taste and smell of Edam cheese when compared to control Edam cheese. The highest texture score was recorded with Edam cheese with higher concentration of BCO (0.6 %) which may reflect the effect of oil in enhancement of cheese texture. In addition, the occurring proteolysis during ripening may affect the texture of cheese depending on the rate of protein breakdown and free up the small peptides and free amino acids.

The BCO also have an obvious effect on the taste of Edam cheese due to its content of organic and volatile fatty acids which may interfere with the pleasant taste of cheese when judged with panelists (Abdel-Razig et al., 2014).Similar observations for the smell development by using some essential oils were found by Ibrahim and Abdel-Hakiem (2015) in Damietta cheese, Ehsani et al. (2016) in Iranian white cheese and Georgescu et al. (2018) in a model food matrix.

Positive correlation was found between BCO andhigh moisture values and prevention of texture deteriorations during the storage of food matrix (Georgescu et al., 2018). The overall acceptability of Edam cheese is shown in Fig. 3, which exhibits the effect of BCO in developing the flavor of Edam cheese.

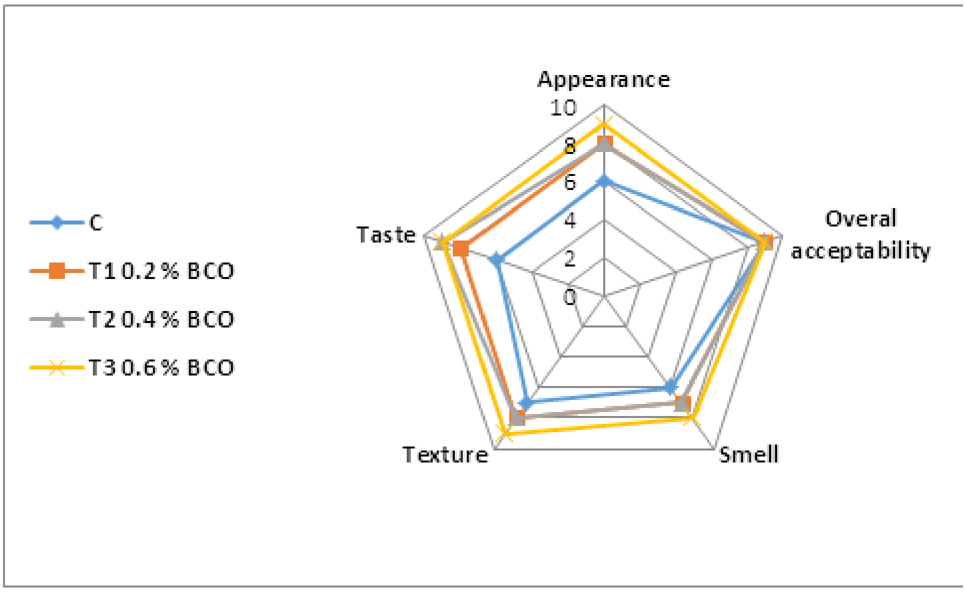

Fig. 3. Sensory score of Edam cheese with different BCO concentrations

Conclusion

The purpose of this study was to evaluate the effects of black cumin oil on the quality characteristics of Edam cheese during ripening. The addition of black cumin oil to Edam cheese affected cheese acidity, the development of ripening indices, free fatty acids and subsequent sensorial score of cheese due to the valuable content of BCO with fatty acids, phenolic compounds and pleasant flavour. Depending on the level of black cumin oil, the total viable count decreased in treated cheese. Coliform bacteria were not detected in cheese with added black cumin oil. The lowest number of yeasts & moulds was 1.00 cfu/g in cheese with 0.6 % BCO. In this study, blackcumin oil had antimicrobial activity. There is no inhibition effect of BCO on the growth of proteolytic bacteria during ripening of Edam cheese. In general, 0.6 % black cumin oil reduced the total viable count, limited the growth of yeasts & moulds, inhibited coliform bacteria and enhanced the growth of proteolytic bacteria. Ripening indices were also higher in Edam cheese with 0.6 % BCO with superior consumer acceptance.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this article.

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تم تحضيرعينات الجبن الإيدام بإضافة تركيزات مختلفة من زيت الحبة السوداء(*Nigella sativa L*) (٠.٢ , ٠.٤ , ٠.٦ ٪ حجم/وزن (. لوحظ أن هناك فروق معنوية ($P \leq 0.05$) فى التركيب الكيميائى وعوامل التسوية بين الجبن الكنترول وعينات الجبن المعاملة (٠.٦ ٪ زيت). أدت إضافة زيت الحبة السوداء إلى زيادةحموضةالجبن من ٠.٧٩٪ في عينة الكنترول إلى ١.١٣٪ في الجبن المعامل بنسبة ٠.٦٪ زيت في بداية فترة التسوية.مع حدوث زيادة مستمرة فى الحموضة في جميع عينات الجبن أثناء التسوية. النيتروجين الذائب/النيتروجين الكلى وصل إلى ١٥.٩١٪ في الجبن المحتوى على نسبة الزيت الأعلى وذلك في نهاية فترة التسوية. سجلت الأحماض الأمينية الحرة ١.٢١ جم من الليوسين/جرام من الجبن في الجبن المحتوى على ٠.٦٪ زيت في نهاية فترة التسوية. زادت الأحماض الدهنية الحرةمع زيادة مستوى الزيت في عينات الجبن الإيدام. أدت إضافة زيت الحبة السوداء في الجبن الإيدام إلى تقليل العد الكلي للبكتريا (5.97 log cfu/g) ,والخمائر والفطريات (1.00 log cfu/g) في نهاية فترة التسوية وحدث تثبيط لنمو بكتريا القولون. سجلت البكتيريا المحللة للبروتين أعداد أعلى (3.19 log cfu/g) ,في حين سجلت البكتيريا المحللة للدهون أعداد أقل (2.59 log cfu/g) في الجبن الإيدم المضاف له ٠.٦٪ من الزيت مقارنة بعينات الجبن الأخرى في عمر ٦٠ يوما من التسوية. تم قبول طعم عينات الجبن المحتوية على تركيز أعلى من زيت الحبة السوداء (٠.٦ ٪) مع عدم وجود أية تعليقات على مظهر ورائحة الجبن. بينما فضل المحكمين بشكل عام الجبن الإيدام المحتوى على نسبة أعلى من الزيت.