



Properties of Cold Pressed Egyptian and Ethiopian Black Seed Oils and Their Impact on Microbiological Quality of Commercially Sold Kariesh Cheese



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Abstract

CHEESE is a common dairy product that can become contaminated by microorganisms, compromising its safety, shelf life, and flavor. Because of their antibacterial qualities, natural preservatives like fixed oils present a possible substitute for synthetic ones. The effects of Egyptian and Ethiopian black cumin (*Nigella sativa* L.) oils on the shelf life and sensory qualities of cheese were examined, as well as its antibacterial activity against common cheese contaminants in relative to the GC-MS chemical profiling. Five fatty acids were identified as saponified components, including palmitic acid (11.94 and 12.06%), oleic acid (22.37 and 21.88%), and linoleic acid (59.84 and 60.13%), as the primary constituents, respectively. While 1,3-bis[(trimethylsilyl)oxy]-propyl stearate (10.19 and 7.20%), 7,3',4'-trimethoxy-quercetin (8.07 and 5.40%), β -sitosterol (30.49 and 25.73%), and stigmaterol (19.71 and 25.62%), were assigned as major un-saponified compounds. The results of evaluating the microbiological quality of commercially sold Kariesh cheese after addition of 0.2% of Egyptian and Ethiopian BCO shows that they were efficient to decrease the total bacterial, Coliforms, *E. coli*, yeast and mold counts contamination in the treated samples. The Ethiopian BCO showed slightly higher antimicrobial effect than Egyptian BCO. This study highlights that BCO is a promising natural preservative for improving the microbiological quality and extending the shelf life of commercially sold cheese.

Key words: Black Seed Oil, Microbiological Quality, Kariesh Cheese, linoleic acid, β -sitosterol, antibacterial activity.

Introduction

The desire from customers for clean labels and ecologically friendly production methods has led to a rise in the application of naturally produced antimicrobial preservatives in the food sector, as opposed to synthetic ones [1, 2]. It is true that artificial food preservatives including formaldehyde, sorbates, nitrates, benzoates, and sulfites have been linked to allergies or cancer [3, 4]. Approximately one-fourth of food losses are caused by microbial

food deterioration [5]. Food waste has a large carbon footprint and costs over \$680 billion in developed nations and \$310 billion in poor nations, based on the Food and Agriculture Organization (FAO) [4, 6]. Furthermore, the likelihood of microbiological contamination by pathogenic bacteria and spoiling is increased by the increasing amount of fresh, minimally processed, and packaged foods [7].

Given its abundance of nutrients, cheese is a perfect medium for the growth of microorganisms.

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Numerous yeasts and fungus, Coliforms, and *Escherichia coli* are common microorganisms causing cheese to deteriorate. Because of their safety and consumers' demand for natural additives, the usage of natural antimicrobials, including essential oils, is becoming more popular [8]. Low-fat cheese has been more popular over the past half-century as consumers, particularly in developed nations, seek to regulate their calorie consumption and lower the prevalence of coronary heart disease, obesity, some types of cancer, and associated morbidity and death [9]. Because it is low in fat, low in salt, high in protein, high in nutrients, and inexpensive, Kariesh cheese has become one of the more common low-fat soft white cheeses in Egypt, especially among the elderly and those with blood pressure and obesity-related conditions [10].

The primary ingredient used in the production of Kariesh cheese is raw milk. It can be prepared with rennet, skimmed cow's or buffalo's milk that has been acid-coagulated, or a combination of the two using natural microbes [11]. The finished product is also offered without a container and uncovered. Consequently, a high frequency of contamination and the development of several unwanted pathogenic and spoiling microorganisms are present, such as molds, yeasts, *Salmonella spp.*, *Escherichia coli*, Coliforms, and Staphylococcus. These microbes can result in food poisoning illnesses and low-quality final products [12, 13].

The food business is looking for new and creative methods of food bio-preservation because of consumers' growing desire for natural and chemical-free products [14]. Spices are utilized as natural preservatives because of their therapeutic benefits as antioxidants and antimicrobials [15]. Some researchers have reported that spices and their derivatives have antifungal, antibacterial, and antioxidant properties [16, 17, 18].

Nigella sativa seeds are used to make black cumin oil (black seed oil: BCO), which has been shown to have potent antibacterial, antioxidant, and anti-inflammatory qualities [19]. The oil or seeds of *Nigella sativa* are used as a lactagogue, vermifuge, carminative, and diuretic [20]. They have been used to add flavor to bread, baked goods, cheese salads, tea, coffee, and other dishes [18, 21]. According to [20], the dried seeds of black cumin contain proteins, alkaloid compounds, 36-38% fixed oils, 0.4-2.5% essential oil, and saponin. Among its many other bioactive qualities, black cumin oil exhibits potent antibacterial, antifungal and antioxidant potentiality [19, 22- 25].

Therefore, the goal of the current study was to use black cumin fixed oil to enhance the microbiological characteristics of commercially available Kariesh cheese in relation to the bioactive components of this oil that were identified using GC-MS analysis.

Material and Methods

Samples collection and chemical analysis of black seed oil (BCO)

Food-grade quality, 100% pure, cold pressed Egyptian BCO and Ethiopian BCO were purchased from Biohayah (Cairo, Egypt). The two oil samples were subjected to saponification with KOH then extracted with *n*-heptane/diethyl ether and finally dried. A 50 μ L of BSTFA was added to the dried extract and incubated in a dry block heater at 70 °C for 30 min. Then, the saponifiable (hydrocarbons) and un-saponifiable (fatty acid methyl esters) samples were subjected to the Gas chromatography–mass spectrometry analysis (GC-MS) analysis according to the experiments below.

GC-MS experiments

The Central Laboratories Network, National Research Centre, Cairo, Egypt, had a GC-MS system (Agilent Technologies) with a mass spectrometer detector (5977A) and gas chromatograph (7890B). The HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μ m film thickness) was installed in the GC. The following temperature program was used for the analyses: 240 °C; increasing at 10 °C/min to 265 °C and held for 1 min; rising at 15 °C/min to 300 °C and kept for 25 min; and utilizing hydrogen as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 10:1. The injection volume was 1 μ L. The temperature of the injector and detector was maintained at 280°C and 290°C, respectively. Electron ionization (EI) at 70 eV was used to obtain mass spectra with a solvent delay of three minutes and a spectral range of m/z 50-550. Quad was 150°C and the mass was 230°C. Spectrum fragmentation patterns were compared to those found in Wiley and NIST Mass Spectral Library data in order to identify various constituents.

Gas chromatography of FAMES

The Agilent Technologies GC model 7890B was fitted with a flame ionization detector at the National Research Center's Central Laboratories Network in Cairo, Egypt. Utilizing a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 μ m film thickness), separation was accomplished. Utilizing hydrogen as a carrier gas, analyses were conducted with a split-1:50 mode, 1.8 ml/min flow rate, 1 μ L

injection volume, and the subsequent temperature program: 3 minutes at 100 °C, followed by a 2.5 °C/min rise to 240 °C and a 10-minute hold. At 250 °C and 285 °C, respectively, the injection device and detector (FID) were maintained.

Microbiological quality of commercially sold Kariesh cheese supplemented with the Egyptian and Ethiopian BCO

Preparation of Kariesh Cheese Samples

Kariesh cheese sample (3 kg) was purchased from a local market at Cairo governorate (at the consumer level). The cheese samples were transferred to the laboratory in an ice box at 7 °C. Cheese samples were thoroughly mixed, and the homogenate was divided into three groups (1 kg gm each) and each group contained three subsamples. The first group was used as a control group (without any oil treatment), the second group was treated with 0.2% Egyptian black cumin oil and the third group was treated with 0.2% Ethiopian black cumin oil. Each group was evenly mixed and stored at 7 ± 1 °C, samples were taken from each group directly after the oil was added (0 day) and then every 3 days and on the 12th day.

Microbiological Analysis of Kariesh Cheese samples

The samples obtained from each group were subjected to microbiological analysis according to ISO methods to determine the total bacterial count, yeast and mold, Coliforms and *Escherichia coli* counts. Colony counts were recorded after incubation at appropriate conditions for each test.

Sensory Evaluation

A panel of 9 trained sensory evaluators assessed the cheese samples for color, texture, aroma, and taste using a standardized scoring system.

Statistical analysis

All tests were carried out in triplicate, and the results were presented as means of standard deviation. The data were analysed using Statistical Software for the Social Sciences (SPSS).

Results and Discussion

Bioactive components of the Egyptian and Ethiopian black cumin seeds oil

GC-MS was used to analyze the golden yellow oils of Egyptian and Ethiopian black cumin seeds that exhibited a powerful fragrant smell. Figure 1 represented the CG-MS chromatograms of the fatty acid components of the Egyptian (Fig. 1A) and Ethiopian (Fig.1B) oils. Additionally, the

hydrocarbon components of the Egyptian (Fig. 2C) and Ethiopian (Fig. 1D) oils were presented.

The two oils were found to contain five main fatty acids, accounting for 100% of the total fatty acid content (Table 1). The identified fatty acids were *cis*-11,14-eicosadienoic acid (2.79 and 2.78%), stearic acid (3.06 and 3.15 %), Palmitic acid (11.94 and 12.06%), oleic acid (22.37 and 21.88%), and linoleic acid (59.84 and 60.13%) were the main constituents of the Egyptian and Ethiopian samples, respectively. According to previous publications [26, 27], the fatty acid content is comparable. The current investigation, oleic and linoleic acids were found as the main fatty acids the Egyptian and Ethiopian oils. Present findings were in complete agreement with the documented data in which the oleic and linoleic acids were found as the main fatty acids in the different oils of black cumin types (> 18.9 and > 47.5%, respectively) [26, 27].

Otherwise, the primary constituents of the Egyptian and Ethiopian oils were 1,3-bis [(trimethylsilyl)oxy]-propyl stearate (10.19 and 7.20%), isofucosterol (6.28 and 6.94%), 7,3',4'-trimethoxy-quercetin (8.07 and 5.40%), β -sitosterol (30.49 and 25.73%), and stigmaterol (19.71 and 25.62%). In the present instance, the high levels of steroids, particularly β -sitosterol, campesterol, and stigmaterol, in the Ethiopian and Egyptian oils were entirely consistent with the data that was previously recorded for the various types of black cumin oils [28, 29, 30].

Impact of Egyptian and Ethiopian BCO on microbiological quality of commercially sold Kariesh cheese

Kariesh cheese is prone to microbial deterioration and pathogens growth due to its very high moisture content and abundance of nutrients. Cheese with high moisture content may allow pathogens to grow quickly and cause spoilage, resulting in color and/or flavor defects that lower the product's quality. Therefore, control of microbial deterioration and pathogens growth are essential. Therefore, this work was planned to build up information on the effect of Egyptian and Ethiopian black cumin (*Nigella sativa* L.) oils on the microbiological and sensory qualities of cheese, as well as its antibacterial activity against common cheese contaminants during cold storage.

The results shown in fig. 2A highlight that there was a significant increase in TBC in non-treated cheese (control) compared with cheese samples treated with 0.2% of either Egyptian or Ethiopian black cumin oil throughout the 12 days of storage period at refrigerator temperature.

Alsawaf and Alnaemi [31] reported that there was a significant decrease ($P < 0.05$) in TBC in Iranian soft white cheese treated with *N. sativa* oil during refrigerated storage.

On the contrary Hassanien et al. [32] observed that adding the BCSO (0.1% or 0.2% w/w) to soft cheese did not produce any significant changes ($p < 0.05$) on the total bacteria and lactic acid bacteria during cold storage.

The presence of Coliforms in milk products indicates the inferior quality of the milk used in manufacture [33]. Fig. 2B shows that addition of Egyptian and Ethiopian BCO was efficient to decrease the Coliforms contamination in the treated samples. The most dangerous type of Coliforms that contaminates milk and milk products is *E. coli*. Although *E. coli* often lives in the intestines of both humans and animals, certain strains are virulent due to pathogenic or toxic virulence genes [13]. Furthermore, *E. coli* is among the main causes of cheese borne food poisoning.

Results shown in figure 2C revealed that Egyptian and Ethiopian BCO has antibacterial effect against *E. coli*, as *E. coli* counts of cheese samples treated with black cumin oil were decrease from the third day of treatment. Nearly similar results were reported by Alsawaf and Alnaemi [31]; Mahgoub et al, [15] and Hassanien et al [32]. The addition of BCO aids in compliance with the Egyptian Standard [34] which recommended that Coliforms count must not be higher than 10 cfu/g.

Cheese has low pH, high moisture content, and nutritional makeup encourage the growth of yeasts and molds. Yeasts have a variety of effects on the safety and quality of dairy products, including the production of alcohol, CO₂, and off-flavors such fermented, yeasty, and fruity flavors[35]. Concerning yeast and mold counts, data reported in fig. 2D pointed out that the means of yeast and mold were decrease markedly during the cold storage. These results revealed that black cumin oil has antifungal activity against fungal contamination in cheese. The Ethiopian BCO showed slightly higher antimicrobial effect than Egyptian BCO (Fig. 2).

Sensory Evaluation

Flavor is the sensation produced by a material taken in the mouth, and it is primarily detected by the senses of taste and smell as well as by the mouth's

general pain, touch, and temperature receptors. Additionally, flavor refers to the culmination of the properties of the substance that generates that feeling [32]. Data reported in table 2 indicated that the addition of BCO did not adversely affect the color, texture, or taste of the cheese. Some evaluators noted a slight aromatic difference in the treated samples, which was generally well-received. Mahgoub et al [15] stated that adding BCSO at levels of 0.1 and 0.2% w/w to Domiati cheese improved its physicochemical and sensory properties. While Hassanien et al [32] observed that there were no clear differences between control and 0.1% BCSO-supplemented cheese in flavor, appearance and texture.

The study demonstrated that BCO possesses strong antimicrobial properties that can effectively reduce microbial contamination in cheese without compromising its sensory qualities. This antimicrobial mechanism of BCO is mainly attributed its high content of bioactive compounds such as thymoquinone, carvacrol, and thymol, which have been shown to disrupt microbial cell membranes and interfere with cellular metabolism [36]. Palmitic acid and oleic acid as main fatty acids were documented to be significant inhibitors of the different bacterial strains [37]. Additionally, the two steroids, β -sitosterol and stigmasterol, along with the flavonoid, 7,3',4'-trimethoxy-quercetin, were reported to have strong inhibitory action against several bacteria including *E. coli* [38].

Conclusion

BCO is a promising natural preservative for improving the microbiological quality and extending the shelf life of commercially sold cheese. Further research is recommended to optimize the concentrations and application methods for industrial use.

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Ethical of approval

Not applicable.

TABLE 1. Bioactive components of the fixed oils of Egyptian and Ethiopian black cumin

No.	Rt	Name	Relative concentrations % (black cumin)	
			Egyptian	Ethiopian
Saponified components (Fatty acids as methyl esters)				
1	24.659	Palmitic acid	11.94±0.13	12.06±0.09
2	30.267	Stearic acid	3.06±0.06	3.15±0.04
3	31.053	Oleic acid	22.37±0.31	21.88±0.24
4	32.784	Linoleic acid	59.84±0.29	60.13±0.32
5	37.621	<i>cis</i> -11,14-Eicosadienoic acid	2.79±0.08	2.78±0.05
Un-saponified components (Hydrocarbons)				
1	3.598	Isochispiin B	0.00±0.00	0.88±0.02
2	3.921	7,3',4'-Trimethoxy-quercetin	8.07±0.08	5.40±0.05
3	4.058	2,3-Bis[(trimethylsilyloxy)propyl] searate	10.19±0.12	7.20±0.07
4	4.092	1-Heptanicosanol	10.12±0.09	3.83±0.11
5	4.596	<i>n</i> -Dotriacontane	0.06±0.00	0.40±0.01
6	4.848	3-Acetoxy-24-methylcholest-8(14)-en-5-ol	1.14±0.03	3.19±0.06
7	5.729	2,2',2'',2'''-Tetramethoxy-5,5',5'',5'''-tetramethyl-1,1',1',1'''-quaterphenyl	1.36±0.04	4.10±0.09
8	6.541	9,19-Cyclolanost-24-en-3-β-acetate	3.90±0.11	5.26±0.08
9	6.713	Campesterol	4.50±0.17	5.05±0.20
10	7.091	Stigmasterol	19.71±0.21	25.62±0.27
11	7.182	Isofucosterol	6.28±0.18	6.94±0.14
12	7.508	β-Sitosterol	30.49±0.25	25.73±0.19
13	7.903	24-Methylene-9,19-cyclolanostan-3-β-ol	1.98±0.08	4.36±0.13
14	8.258	Digitoxin	0.43±0.02	0.72±0.01
15	8.636	Betulin	0.44±0.01	0.15±0.00
16	9.013	1,25-Dihydroxyvitamin D3	1.26±0.03	1.14±0.06
17	12.555	1-Monolinoleoylglycerol	0.07±0.00	0.03±0.00

Rt: retention time; All the components were characterized in their trimethylsilyl (TMS) derivative

TABLE 2. Sensory characteristics of cheese supplemented with Egyptian and Ethiopian BCO

Treatments	Flavor (50)			Body and texture (40)			Appearance (10)			Total (100)							
	0 day	3 rd day	6 th day	0 day	3 rd day	6 th day	0 day	3 rd day	6 th day	0 day	3 rd day	6 th day					
Control	46.5	45	44	43	37	35.5	34	33	33	8.5	8.5	8.0	7.5	92	89	86	84
Egyptian BCO	46.5	45	44	44	37	34	33.5	33	33	8.5	8	7.5	7.5	92	87	85	85
Ethiopian BCO	46.5	45	44	44	37	34	33.5	33	33	8.5	8	7.5	7.5	92	87	85	85

Each value is the mean of three replicates

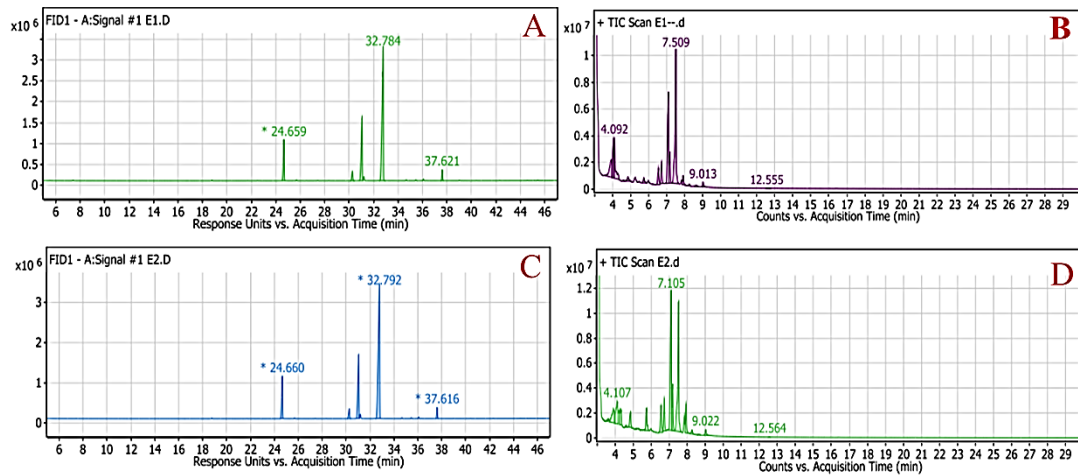


Fig. 1. GC-MS of saponified (A and C) un-saponified (B and D) components of the Egyptian and Ethiopian black cumin seeds oil, respectively.

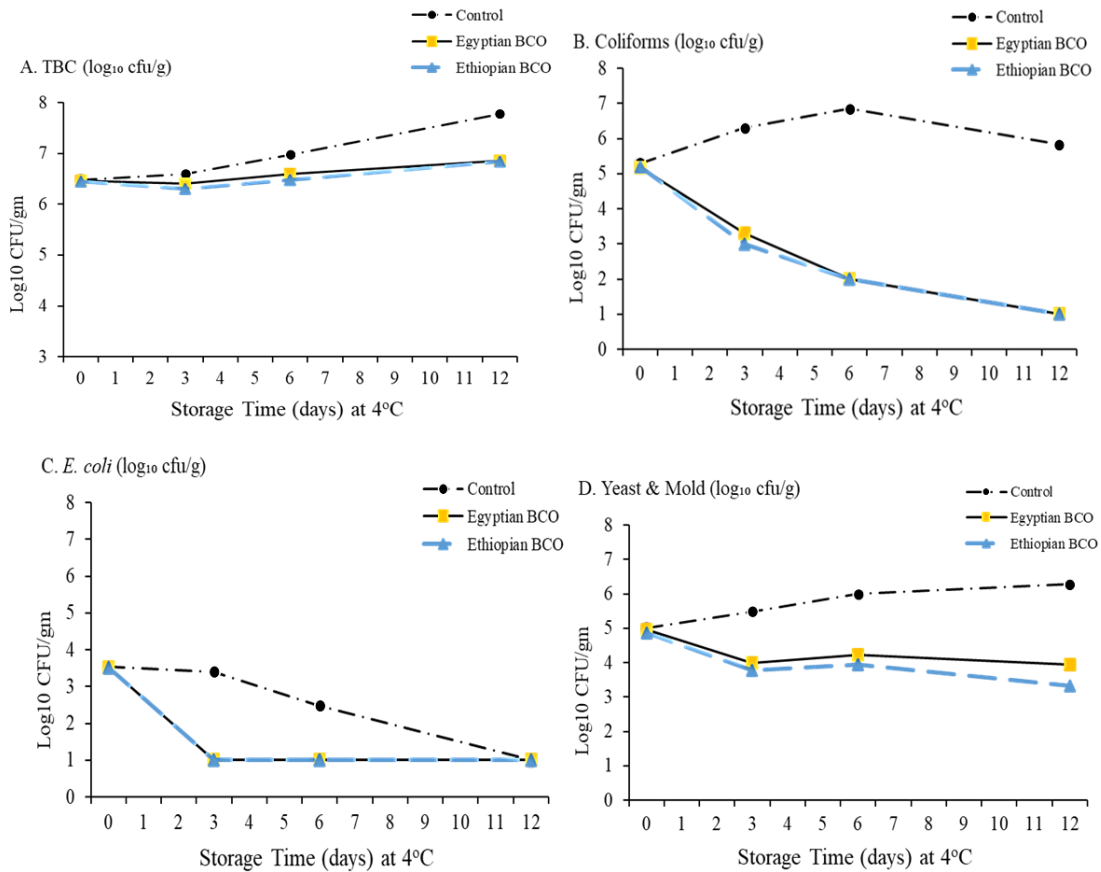


Fig. 2. Effect of the cold pressed Egyptian and Ethiopian BCO on the means of A: TBC, B: Coliforms, C: *E. coli* D: Yeast and Mold during storage of Kariesh cheese at refrigerator temperature (7 ± 1 °C)

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المكونات الكيميائية لزيوت الحبة السوداء المصرية والإثيوبية المعصورة على البارد وأثرها على الجودة الميكروبيولوجية للجبن القريش المباع تجارياً

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الملخص

الجبن هو أحد منتجات الألبان الشائعة التي يمكن أن تتلوث بالكائنات الحية الدقيقة، مما يعرض سلامتها ومدة صلاحيتها ونكهتها للخطر. نظراً لخصائصها المضادة للبكتيريا، فإن المواد الحافظة الطبيعية مثل الزيوت الثابتة تقدم بديلاً محتملاً للزيوت الاصطناعية. تم فحص تأثيرات زيوت الكمون الأسود المصري والإثيوبي (حبة البركة) على العمر الافتراضي والصفات الحسية للجبن، بالإضافة إلى نشاطه المضاد للبكتيريا ضد ملوثات الجبن الشائعة بالنسبة للتنميط الكيميائي MS-GC. تم تحديد خمسة أحماض دهنية كمكونات صابونية، بما في ذلك حمض الألميتيك (11.94 و 12.06%)، وحمض الأوليك (22.37 و 21.88%)، وحمض اللينوليك (59.84 و 60.13%)، كمكونات أساسية، على التوالي. بينما تم تعيين 1،3-مكرر [ثلاثي ميثيل سيليل] أوكسي] -جروبيل ستيرات (10.19 و 7.20%)، 7،3'، 4-تريميثوكسي-كيرسيتين (8.07 و 5.40%)، β -سيتوستيرول (30.49 و 25.73%)، وستيجماستيرول (19.71 و 25.62%)، كمركبات رئيسية غير صابونية. أظهرت نتائج تقييم الجودة الميكروبيولوجية للجبن القريش المباع تجارياً بعد إضافة 0.2% من BCO المصري والإثيوبي أنها كانت فعالة في تقليل إجمالي التلوث البكتيري والقولوني والإشريكية القولونية والخميرة والعفن في العينات المعالجة. أظهر BCO الإثيوبي تأثيراً مضاداً للميكروبات أعلى قليلاً من BCO المصري. تسلط هذه الدراسة الضوء على أن BCO مادة حافظة طبيعية واعدة لتحسين الجودة الميكروبيولوجية وإطالة العمر الافتراضي للجبن المباع تجارياً. الاستخدام.

الكلمات الدالة: زيت الحبة السوداء (BCO)، الجودة الميكروبيولوجية، الجبن القريش، حمض اللينوليك، β سيتوستيرول، نشاط مضاد للبكتيريا.