The Effect of Alginate Microcapsules of Linseed and Black Seed Oil on the Microbiological and Sensorial Properties of Chocolate Ganache During Storage

Mohimani, Banafshe; Safaeian, Shila*[*+]*; Mousavi Nadoshan, Rezvan; Rabbanim, Mohammad

Department of Food Science and Technology, Faculty of Biological Science, North Tehran Branch, Islamic Azad University, Tehran, I.R. IRAN

Tavakolipour, Hamid

Department of Food Science and Technology, Faculty of Agriculture, Sabzevar Branch, Islamic Azad University, Sabzevar, I.R. IRAN

ABSTRACT: *The application of bioactive natural oils in the food industry is limited due to their instability and poor solubility in hydrophilic media. This study aimed to investigate the effect of alginate microcapsules of linseed and black oil on microbial and sensorial properties of chocolate ganache during 28 days of storage. The results showed that both evaluated oils had considerable antibacterial effects against tested microorganisms (Salmonella Typhimurium, Escherichia coli, Staphylococcus aureus, Aspergillus niger, and Candida albicans) and gram-negative than gram-positive bacteria were more resistant to linseed oil. Therefore, Black seed oil showed higher antibacterial activity and both linseed and black seed oil microcapsules had lower antibacterial effects than their free form. The incorporation of encapsulated oils in chocolate ganache was not significantly different from the control samples. Although, the free form has shown higher antibacterial activity, due to the characteristics of protecting the bioactivity of oils from undesirable conditions, controlled release, and marketability of the product, loading the oils in an alginate bead is a suitable way for the application of black seed and linseed oil in food products.*

KEYWORDS: *Ganache; Antibacterial; Linseed oil; Black seed oil; Alginate bead.*

INTRODUCTION

In recent years, the increment of public awareness about the relationship between diet and health has led to increased demand for functional foods that exhibit health-promoting properties [1]. Moreover, the disadvantages of synthetic food preservatives have resulted in the growing interest of researchers to find and identify natural compounds with bioactive properties. Among different common foods, chocolate is a popular treat that provides consumers pleasure and is consumed increasingly all over the world. Ganache is made from dairy and chocolate and

[*] *To whom correspondence should be addressed.*
+ E-mail: Shilasafaeian6@gmail.com
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has a wide application in confectionery products such as cakes, tarts, and cookies [2]. The use of preservatives is essential in the chocolate confectionery industry to warrant microbial safety. As mentioned, the increase in consumer demand for functional foods and natural additives has resulted in a great need for green additives (natural compounds with preservative activity). It has been reported that the antimicrobial activity of EOs can be attributed to the presence of a number of low molecular weight phenols, terpenes and aldoketones, which also have been shown to exhibit antimicrobial activity in pure form. A number of compounds in relatively low concentrations, such as α-pinene, β-pinene, thymol, γ-terpinen, terpinolene, piperitone and perillene, could also be expected to make a significant contribution to the antimicrobial activity of the Eos. Essential oils could lead to leakage of proteins of pathogen cells. These compounds showed disruptive action on cell membrane, as well as the content and activity of bacterial proteins. Also, the activity of endo-enzymes decreased after treatment. Essential oils could affect the HMP Pathway of *E. coli* and decrease the activity of the key enzyme (G6PHD). They also could affect the EMP Pathway of *S. aureus* and decrease the activity of three key enzymes (PFK, HK and PK). Among natural bioactive compounds with the potential to be used in Ganache, polyunsaturated fatty acids (PUFA) are suitable choices that have considerable effects on human health such as the prevention of hypertension and cardiovascular diseases [3]. The n-3 fatty acids family, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and α-linolenic acid (ALA), is one of the most important sources of PUFAs which is abundant in vegetable oils especially linseed oil [4]. Linseed (*Linum usitatissimum*) contains a high amount of oil (about 40%), of which about 90% is PUFA. The essential fatty acid of α-linolenic acid is the main component of linseed oil PUFAs with a content of more than 50% [5]. The linseed oil has health-beneficial properties such as anti-cancer, cardiovascular, chronic inflammation, and improvement of brain functions [6]. Also, it has been reported that flaxseed oil has considerable antibacterial activity against various pathogens because it contains a high amount of secoisolariciresinol diglucoside (SDG), the precursor of lignans, which exerts antibacterial potentials [7]. On the other side, the Black seed (*Nigella sativa*) is a flowering plant from the *Ranunculaceae* family**.** The black seeds contain 36-38% oil, proteins, alkaloids, and essential oils. In addition, dithymoquinone, thymohydroquinone, thymol, niglimine-N-oxide, carvacrol, alphahedrin, niglidin and thymoquinone compounds have also been isolated from black seed oil. [8, 9]. Different studies showed the high therapeutic and health-beneficial potential of black seed oil such as antihistaminic, anti-diabetic, cholesterol-lowering, antibacterial, anti-inflammatory, and antihypertensive activity [10]. According to the above-mentioned issue, linseed and black seed oils have the potential to be used as an antibacterial agent in chocolate ganache. Nevertheless, their application in hydrophilic food systems is limited due to their susceptibility to oxidative rancidity, poor oral bioavailability, and insolubility in water [11]. Encapsulation is an effective solution to overcome these challenges. Different carrier systems have been used for the encapsulation of bioactive compounds such as lipid carriers, niosomes, emulsions, and hydrogels [12]. Hydrogels are swellable polymer networks that are suitable for the encapsulation of bioactive compounds such as plant oils. Among different polysaccharides, Alginate [13] is the most common polymer that has been used widely in the production of hydrogels due to its suitable properties such as biocompatibility, non-toxicity, stability, and easy production process [14]. So, using food-grade hydrogel beads is a promising solution for applying natural bioactive compounds such as plant oil in food systems. Different studies have applied alginate hydrogels for the encapsulation of bioactive compounds such as curcumin [15], linseed oil, black seed oil [13], and tea polyphenols [16]. Besides, various studies have been reported by *Muthukumarasamy* and *Holley* (2006) [17], *Malmo et al*. (2013) [18], and *Ghasemnezhad et al*. (2017) [19] regarding the use of alginate beads filled with bioactive compounds in dry fermented sausage, chocolate soufflé, and chocolate milk, respectively.

To our knowledge, there is no study considering the effect of linseed and black seed oil loaded in alginate microcapsules on microbial counts of chocolate ganache. So, this study aimed to encapsulate linseed and black seed oil in alginate beads and apply the loaded beads as an antibacterial agent in chocolate ganache.

EXPERIMENTAL SECTION

The linseeds and black seeds were purchased from the local market of Boroujen, Chaharmahal and Bakhtiari Province, Iran. Sodium alginate was obtained from Sigma-

Aldrich. All other chemicals and reagents were acquired from Merck Company.

Oil extraction

The linseeds and black seeds were ground and sieved to the particle size of 2-5mm. The oil extraction process was done by a soxhlet apparatus at 50 °C for 5 h by using petroleum ether as solvent. At the end of the process, the solvent was evaporated under a vacuum at room temperature. The resulting oil was kept in a dark bottle at -18 °C until use [20]. The oil extraction yield was determined with the following equation:

$$Extraction\ yeild\ (\%) = \frac{w_f}{w_i} \times 100 \quad (1)$$

Where, w_i is the mass of linseeds before extraction and w_f is the mass of extracted oil.

Physicochemical properties

The acidity, saponification, refractive index, and peroxide value of linseeds and black seeds were determined according to AOAC methods [21]. Also, The p-anisidine value was measured based on AOCS method [22].

Gas chromatographic analysis

Fatty acid compositions of linseed and black seeds were evaluated by Gas Chromatography (GC). Briefly, fatty acid methyl esters (FAME) were prepared by saponification by 0.5 M NaOH–MeOH and methylation by BF_3–MeOH (14%). The FAMEs samples were injected into the gas chromato graph (YL Instrument 6500 GC system) equipped with a flame ionization detector and capillary column (60 m×0.32 mm×0.20 µm). Nitrogen, as the carrier gas, was used at a constant flow rate of 1.0 mL/min. The temperature program was: the initial temperature of 50°C for 2 min, then the temperature reached 140°C by the rate of 4°C/min and was held for 40 min, after that the temperature raised to 210°C and was held for 8 min. The injector and detector temperatures were 250°C and 280°C, respectively [23].

Encapsulation of extracted oils

Emulsion preparation

Briefly, sodium alginate and corn-resistant starch were dissolved in distilled water in the concentration of 3% and 2%, respectively. The mixing was continued for 24 h. Then, the resulting solution was mixed with linseeds or black seeds (3%) and 0.2% of Tween 80 and homogenized for 3 min by using an Ultra Turrax (IKA T25, Germany) at 11000 rpm [11].

Alginate beads preparation

The preparation of alginate beads was carried out by the method of Chan (2011) [24]. 5 mL of the emulsion was dripped into $CaCl_2$ solution (1.5% w/v) through a needle (0.25 mm). The distance between the $CaCl_2$ solution and the needle was 5.0 ± 0.1 cm. After that, to harden the generated beads, they were maintained in $CaCl_2$ solution for 10 min. Finally, the beads were collected by using a sieve and washed two times with distilled water. The obtained beads were kept at 4 ± 1°C until further analysis.

Emulsion characterization

Light microscopy (Lab o med lx 400, U.S.A) in 40x magnification applied for visualization of droplet oil in the alginate solution [25].

Morphology and particle size of the microcapsules

The mean diameter of 100 randomly selected beads was measured from the images capture by digital microscope (AM7013MZT Dino-Lite Premier, Taiwan) using ImageJ software (version 1.51f, NIH, Maryland, USA) [26].

Antimicrobial activity

Microbial strains

The evaluated Microorganisms in this study were: *S. Typhimurium* (PTCC: 1761), *E. coli* (PTCC: 1769), *S. aureus* (PTCC: 1337), *A. niger* (PTCC: 5154) and, *C. albicans* (PTCC: 5027) were obtained from Pasture Institute (Iran).

Determination of Minimum Inhibition Concentration (MIC)

MIC of free and encapsulated linseeds and black seeds was evaluated by serial dilution method. Briefly, Serial dilutions of free and encapsulated linseeds and black seeds were prepared in the range of 1-4% and added to sterile test tubes containing Mueller–Hinton broth (MHB). Then, Bacterial strains (1.5×10^8 CFU/mL, 0.5 McFarland's standard) were inoculated and the tubes were incubated at 37°C for 24 h. The MIC value was the lowest concentration used for free and encapsulated linseeds or black seeds without visible growth in the test tubes [27].

Determination of Minimum Bactericidal Concentration (MBC)

After MIC analysis, 50 µl of all tubes that showed no growth of microorganisms were transferred to Mueller-Hinton agar (MHA) and incubated at 37°C for 24 h. MBC value was the lowest concentration of free and encapsulated linseeds or black seeds which resulted in the killing of 99.9% of the microbial population [27].

Chocolate ganache preparing

In order to prepare the control sample, first, whipped cream (100 grams) was heated in a pot and as soon as it boiled, butter and chocolate were added to the pot while stirring. After mixing and melting the chocolate and butter completely, the flame was turned off. After mixing thoroughly for 1 minute, the obtained mixture was transferred to molds containing chocolate shell and covered with chocolate. The molds were stored in the refrigerator [28]. The treatments were as follows: no treatment (control), without preservative inoculated with 10^5 (CFU/mL) microorganisms (GM), with 3% linseed oil inoculated with 10^5 (CFU/mL) microorganisms (GOM1), with 3% encapsulated linseed oil inoculated with 10^5 (CFU/mL) microorganisms (GEOM1), with 3% black seed oil inoculated with 10^5 (CFU/mL) microorganisms (GOM2), with 3% encapsulated black seed oil inoculated with 10^5 (CFU/mL) microorganisms (GEOM2).

Antimicrobial activity of extracted oils in chocolate ganache

After 72 h of storage at 25°C, each sample time was pummeled with 100 mL of 0.1% sterile peptone water in a Stomacher (2 min). Then, the proper dilutions were spread on each pathogen-specific agar. After incubation for 24 h at 37°C, the pathogens viable cell counts were done.

Sensorial evaluation

The sensorial evaluation was performed using 30 staff from the Farmand factory (Tehran, Iran). Samples were evaluated for texture, appearance, odor, and overall acceptability at 0, 7, 14, 21, and 28 days of storage. A 9-point hedonic scale was used for estimation of the sensory score, where 1=extremely unfavorable and 9=extremely favorable.

Statistical analysis

Statistically, the analysis of data was performed by one-way ANOVA using SPSS 16.0 software. Duncan's test was used for the comparison of means at a 95% level of significance. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Physicochemical properties and extraction yield

The physicochemical properties and extraction yield of linseed and black seed oil are provided in Table 1. The extraction yield of linseed oil was 27.73±0.17% which was different from the reported value by *Ivanov et al.* (2012) [29] and *Kasote et al*. (2013) [30] who revealed the extraction yield of linseed oil amounts of 41.28% and 31.9 %, respectively. *The* extraction yield of black seed oil was 25.5±0.12% which was lower than the extraction yield stated in studies by *Mohammed et al*. (2016) [31] and *Khoddami et al*. (2011) [32]. These different results can be related to the differences in extraction method, type of the seeds, and the used solvents. The variables of saponification, acidity, and iodine content are considered as a criterion of the average molecular weight, the content of free fatty acids, and the degree of unsaturation of the oil, respectively. In this study, the linseed oil oxidative values were in the range of standard values reported by *Nykter et al.* (2006) [33]. The acidity and refractive index of extracted black seed oil were not significantly different from the values reported by *Kiralan et al.* (2014) [34], but their reported peroxide value was markedly higher than calculated value in this study. These results indicate the proper conditions of the oil extraction process, which has led to the production of linseed and black seed oil with acceptable oxidative stability.

GC analysis

The fatty acid compositions of linseed and black seed oil are given in Table 2. Linseed oil has a great fatty acid profile, and its major component is usually α−Linolenic acid (about 60%) [35]. In this study, α-linolenic acid (47.1%) in flaxseed oil and linoleic acid (57.7%) in black seed oil was the main fatty acid component. On the other side, the lowest fatty acid content of linseed and black seed oil belonged to Myristoleic acid (0.06%) and Palmitoleic acid (0.1%), respectively. Different fatty acid compositions were reported for linseed oil; for example, *Zhang et al.* (2008) [23] evaluated the fatty acid composition of flaxseed oil from Hebei Province of China. They reported that the highest fatty acid content was related to α−Linolenic acid (55.78%) and the level of

Table 1: Physicochemical properties and extraction yield of linseed and black seed oil

Parameters	Average value of linseed oil	Average value of black seed oil
Acidity (mg KOH/g)	5.15 ± 0.12	9.68±0.05
Peroxide value (meqO_2/kg)	0.093 ± 0.005	2.66±0.03
Iodine value (gI_2/100g oil)	99.53 ± 0.25	103.46±0.35
Refractive Index (n^{20}_D)	1.46 ± 0.002	1.47±0.001
Saponification value (mg KOH/g)	178.27 ± 0.47	183.96±0.98
P-anisidine value	2.49 ± 0.04	2.8±0.05
Extraction yield (%)	27.73 ± 0.47	25.5±0.12

Table 2: Fatty acid composition of linseed and black seed oi

No		Fatty acid	Average value (% of total fatty acids)	
			Linseed oil	Black seed oil
1	Lauric acid	C12:0	0.1 ± 0.00	0.0±0.0
2	Myristic acid	C14:0	0.1 ± 0.10	11±1.1
3	Myristoleic acid	C14:1	0.06 ± 0.06	0.0±0.0
4	Palmitic acid	C16:0	12.7 ± 0.32	12.5±0.36
5	Palmitoleic acid	C16:1	0.1±0.0	0.1±0.0
6	Stearic acid	C18:0	1.5 ± 0.06	3.2±0.15
7	Oleic acid	C18:1	12.2 ± 0.11	25.7±0.49
8	Linoleic acid	C18:2	19.17 ± 0.06	57.7±0.1
9	α−Linolenic acid	C18:3	47.1 ± 0.1	0.3±0.0

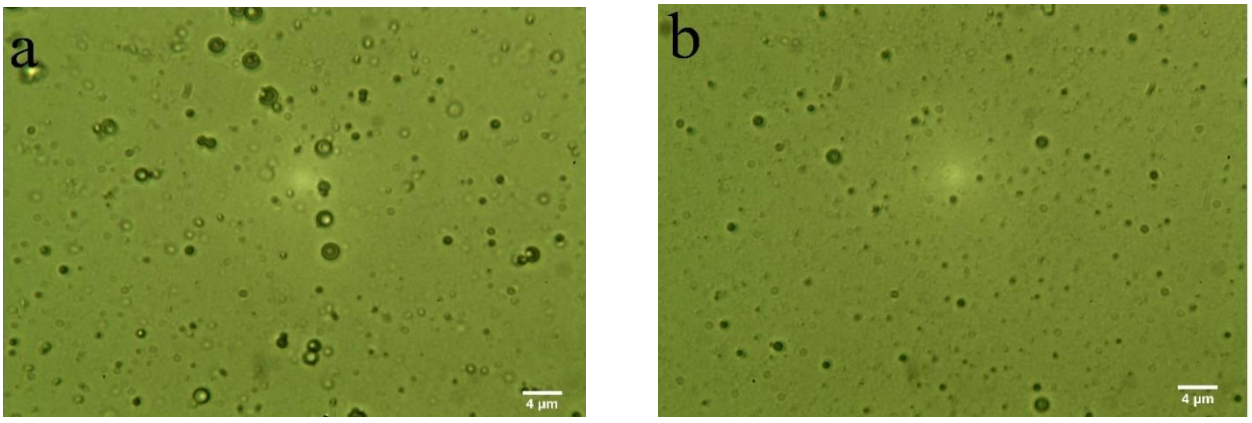

Fig. 1: Distributions of droplet of linseed (a) and black seed oil (b) in the alginate solution

linoleic acid and oleic acid were 15.81 and 16.76%, respectively. On the other side, *Bean* and *leeson* (2002) [36] revealed that the average content of α−Linolenic acid, linoleic acid, and oleic acid was 57.11%, 14.44%, and 18.50%, respectively. In the regard to black seed oil composition, similar linoleic acid values were assessed by *Kiralan et al.* (2014) [37] and *Ramadan* and *Morsel* (2004) [38]. But, lower linoleic acid values then this study were reported by *Kiralan et al.* (2012) [39] and *Cheikh-Rouhou et al.* (2007) [40]. It can be inferred that these different results can be related to the differences in analysis procedures, spices, growth, and geographical conditions.

Emulsion characterization

Fig. 1 shows the distribution of linseed and black seed oil droplets in alginate solution. Considering the droplet size, it can be interpreted that black seed oil droplet is smaller compared to linseed oil and appears uniformly

Fig. 2: Morphology of alginate hydrogel beads containing linseed and black seed oil (a,b) and empty hydrogel as control sample (c)

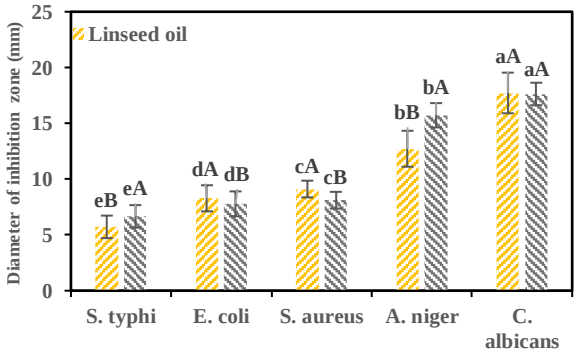

Fig. 3: The diameter inhibition zone of linseed and black seed oil against microorganisms

with a narrow size distribution. The smaller droplets and more uniform distribution of black seed compared to linseed oil may be related to the fatty acid profile of the oil, which affected its performance properties in emulsification [41, 42].

Morphology and particle size of the microcapsules

Morphology characteristics of alginate hydrogel beads containing linseed and black seed oil in addition with empty hydrogel as control sample shown in Fig. 2. Particle size of the hydrogel beads containing linseed and black seed oil calculated 771.165±47.88 and 732.18±36.05 µm respectively and for empty hydrogel 782.8±22.4 µm obtained. Considering the particle size calculated for different alginate hydrogel beads containing oil and comparing the results with empty hydrogel, insignificant difference in particle size was observed. The slightly smaller particles in the oil-containing bead may be related to the modulation of surface tension as a result of the addition of surfactant to the alginate solution to prepare the emulsion. The particle size obtained for the emulsion-filled alginate beads is similar to that reported in the past literature [43, 44].

Antibacterial activity of free linseed and black seed oil

Some foodborne pathogens such as *S. typhi* and *S. aureus* are known for their resistance to antibiotics so discovering natural antibacterial compounds against them is very important [45]. Free linseed and black seed oil showed considerable antibacterial effects against tested microorganisms. Both linseed and black seed oil showed the highest and lowest antibacterial activity against *C. albicans* and *S. typhi*, respectively. The diameter inhibition zone of *C. albicans* in response to linseed and black seed oil was 17.72 and 17.63 (mm) (Fig. 3). On the other side, the diameter inhibition of linseed oil against *S. typhi* was significantly lower than black seed oil ($p<0.05$), which shows the stronger antibacterial effect of black seed oil. The differences in inhibition activity of evaluated oils can be due to their chemical composition differences, extraction condition and the synergistic effect of their minor and major components [46]. The antibacterial activity of black seed oil in various studies reported by *Mohammed et al*. (2019) [47] and *Nair et al*. (2005) [48]. The antibacterial activity of black seed oil is due to its bioactive components such as thymoquinone (TQ), thymol, carvacrol, and etc. In particular, TQ is the most important compound responsible for the antibacterial activity of black seed oil. Because TQ is so flexible, it can change shape, pass through the cell wall of bacteria and kill them [49]. Generally, both linseed and black seed oil had stronger antibacterial activity against gram-positive bacteria (*S. aureus*) than gram-negative bacteria (*E. coli* and *S. typhi*). Similar to our results, *Al-Mathkhury et al*. (2016) reported that the linseed oil showed weak antibacterial activity against *E.coli* as a gram-negative bacteria [50]. The higher antibacterial effect of bioactive compounds such as linseed and black seed oil against gram-positive bacteria can be due to the differences in the structure of their cell membrane [51]. Gram-negative bacteria have an outer membrane on their cell membrane in addition

Table 3: Antibacterial activity (MIC and MBC) of free linseed and black seed oil by the microdilution method

	Free linseed oil		Free black seed oil	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
S. typhi	34.00 ± 5.14 ^{Db}	39.65 ± 2.40 ^{Ab}	34.77±4.90^{Cb}	38.16±3.03^{Bb}
E. coli	38.72 ± 5.35 ^{Ca}	43.83 ± 6.15 ^{Aa}	35.01±5.14^{Da}	39.19±5.34^{Ba}
S. aureus	30.55 ± 2.35 ^{Cc}	36.00 ± 5.16 ^{Ad}	28.33±2.31^{Dd}	33.33±1.41^{Bd}
A. niger	31.44 ± 5.11 ^{Dd}	38.01 ± 4.19 ^{Ac}	32.12±6.33^{Cc}	37.19±7.40^{Bc}
C. albicans	27.77 ± 8.08 ^{Ce}	32.78 ± 7.80 ^{Ae}	26.66±7.98^{De}	31.32±6.53^{Be}

Different capital and small letters show significant differences in rows and columns, respectively ($p<0.05$)

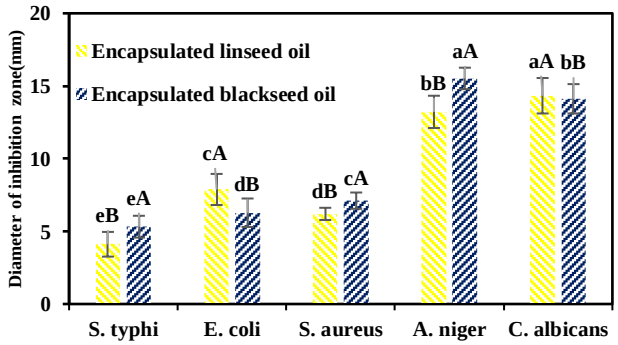

Fig. 4: The diameter inhibition zone of microencapsulated linseed and black seed oil against microorganisms

to the peptidoglycan layer. The lipopolysac-charide molecules present on the surface of this membrane act as barriers against the penetration of the antibacterial compounds. Furthermore, there are enzymes in their periplasmic space that can break down the foreign molecules from outside the membrane [52].

The MIC and MBC of free linseed and black seed oil are shown in Table 3. The MIC values of free linseed and black seed oil were in the range of 27.77- 38.72 and 26.66-35.01 (µg/mL) and as predicted, the MBC values against all tested microorganisms were higher than MIC values. On the other hands, both free linseed and black seed oil had the strongest antibacterial activity against *C.albicans* with the lowest MIC value of 27.77 and 26.66 (µg/mL), respectively. The most resistant bacteria against free linseed and black seed oil was *E.coli*. It can be concluded that the more resistance of gram-negative bacteria (*S. typhi* and *E.coli*) to tested oils can be related to the outer membrane of gram-negative bacteria which covered the peptidoglycan layer. Lipopolysaccharide molecules on this membrane with its hydrophilic property increase the resistance of gram-negative bacteria against antibiotic compounds. On the other hand, in gram-positive bacteria, antibacterial compounds easily affect the cytoplasmic membrane. [52]. Generally, the antibacterial potential of linseed oil is attributed to the lignans precursor, SDG, which has health beneficial effects on human health. Also, the antibacterial activity of back seed oil is mostly related to the TQ [49]. The antibacterial effect of linseed and black seed oil against *E. coli* and *S. aureus* was reported by Amin and Thakur (2014) [53] and *Georgescu et al*. (2018) [54]. On the other side, the anti-fungal activity of linseed oil was revealed by *Adolphe et al*. (2010) [55]. Contrary to our findings, *Al-Mathkhury et al*. (2016) [50] reported that flaxseed oil did not have a significant effect on *E.coli*. These differences can be due to the used concentration of linseed oil, the condition of the experiment, and the extraction process.

Antibacterial activity of encapsulated linseed and black seed oil

According to obtained results, both encapsulated linseed and black seed oil showed acceptable inhibition effects against tested microorganisms. The encapsulated linseed oil had a higher antibacterial effect against *E. coli*, and *C. albicans* than encapsulated black seed oil while encapsulated black seed oil showed higher activity against *S. typhi*, *S. aureus*, and *A. niger* than encapsulated linseed oil. The antibacterial effect of encapsulated linseed oil against microorganisms was in the order of *C. albicans*> *A. niger*> *E. coli*> *S. aureus* > *S. typhi* while the anti-bacterial effect of black seed oil was in the order of *A. niger*> *C. albicans*> *S. aureus* > *E. coli* > *S. typhi* (Fig. 4). These findings show the high resistance of gram-negative bacteria such as *E. coli* and *S. typhi* against antibacterial compounds which are attributed to the outer protectant layer on their membrane [52]. These results are consistent with the findings of *Joshi et al*. [56] who demonstrated that linseed oil had a less antibacterial effect against *E.coli* as a gram-negative bacteria than *S.aureus* as a gram-positive bacteria.

Table 4: Antibacterial activity (MIC and MBC) of encapsulated linseed oil by the microdilution method Different capital and small letters show significant differences in rows and columns, respectively (p<0.05)

	Encapsulated linseed oil		Encapsulated black seed oil	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
S. typhi	37.06 ± 0.24^{Bb}	43.35 ± 0.15^{Ab}	36.25 ± 0.12^{Db}	40.11 ± 0.10^{Cb}
E. coli	40.72 ± 0.30^{Ca}	47.20 ± 0.18^{Aa}	38.34 ± 0.20^{Da}	42.88 ± 0.13^{Ba}
S. aureus	35.25 ± 0.12^{Cd}	41.75 ± 0.02^{Ac}	31.96 ± 0.21^{Dd}	36.20 ± 0.45^{Bd}
A. niger	35.96 ± 0.01^{Cc}	40.21 ± 0.17^{Ad}	34.73 ± 0.71^{Dc}	39.20 ± 0.26^{Bc}
C. albicans	29.66 ± 0.08^{Ce}	35.47 ± 0.16^{Ae}	29.61 ± 0.72^{De}	32.03 ± 0.62^{Be}

MIC and MBC of encapsulated linseed and black seed oil

As shown in Table 3. The encapsulated form of linseed and black seed oil had considerable antimicrobial effects against evaluated microorganisms. Also, the MIC and MBC of encapsulated linseed oil were significantly higher than the values of encapsulated black seed oil ($p<0.05$), which shows the stronger antimicrobial activity of black seed oil than linseed oil. These results show the protective effect of encapsulation with alginate beads on the retention of antibacterial activity of encapsulated oils. In line with our study, *Torpol et al*. (2019) reported that hydrogel beads containing garlic and holy basil essential oils had an efficient antibacterial effect [57]. On the other side, both fungi showed the most sensitivity to the encapsulated oils. For example, the lowest MIC value against both encapsulated linseed and black seed oil was related to *C. albicans* in the amounts of 29.66 and 29.61 (µg/mL). Similar to our results, *Cox et al*. (2000) and *Zineb*, and *Yacine* (2018) revealed that the fungi were more sensitive than bacteria to the essential oil of tea tree oil, and orange and lemon, respectively [58]. Also, *Nzeako et al*. (2006) [59] demonstrated that *C. albicans* had the lowest resistance to clove oil and thyme to *E. coli*, *S. aureus*, and *Salmonella* species. On the other side, *E.coli* showed the most resistance against both encapsulated linseed and black seed oil.

The considerable antimicrobial activity of encapsulated oil against tested microorganisms maybe is due to the interaction of encapsulated oils with the cell membrane of the microorganisms which alters its permeability and leads to the death of the cells [60].

Comparison of the antibacterial effect of free and encapsulated linseed oil

A comparison of the results of Tables 2 and 3 show that both free linseed and black seed oil were more effective against microorganisms than encapsulated form, due to MIC and MBC of free form were lower than encapsulated form. For instance, the MIC of encapsulated and free linseed oil against *C. albicans* was 29.66 (µg/mL) and 27.77 (µg/mL). This can be attributed to the sustainable and slow release of flaxseed oil from bead hydrogels in comparison to its free form [61]. Also, the lower antibacterial activity of encapsulated oils can be due to the use of an ultrasound probe for emulsion preparation which may damage the oils bioactive compounds. In line with our study, *Radünz et al*. (2019) reported that the antibacterial activity of clove essential oil encapsulated in alginate bead was lower than its free form [60]. In contrast to these results, *Hashim et al*. (2019) reported that encapsulated flaxseed oil had a more antibacterial effect against *E.coli* and *S.aureus* [62]. Contradictory results can be related to the differences in encapsulation efficiency, encapsulation methods, and the used wall materials. Also, The interactions between loaded bioactive compounds and wall materials can affect their bioactivity.

Antimicrobial activity of linseed and black seed oil in chocolate ganache

The effect of linseed and black seed oil in a free and encapsulated form on chocolate ganache after 72 h of storage at 25°C is shown in Table 5. *S. typhi*, *A. niger*, and *C. albicans* were not identified in the control sample and the most predominant microorganisms in the control sample were *S. aureus* and *E. coli*. The incorporation of both linseed and black seed oil in free form was more effective in the growth inhibition of microorganisms in comparison to encapsulated oils. For instance, viable counts of *E. coli* in GOEM 1 were 3.17 (Log CFU/g), while its counts were 3.01 (Log CFU/g) in GOM 1 treatments. On the other side, viable counts of *E. coli* in GOEM 2 were 3.47 (Log CFU/g) while in GOM 2 was

Table 5: Antimicrobial activity of free and encapsulated linseed oil in chocolate ganache

	Control	GM	GOM 1	GOM 2	GEOM 1	GEOM 2
S. typhi	-	+	-	-	-	-
E. coli	0.52^{Fb}	3.11^{Cb}	3.01^{Da}	2.84^{Ea}	3.17^{Ba}	3.47^{Aa}
S. aureus	1.07^{Fa}	3.25^{Aa}	2.24^{Db}	2.14^{Eb}	2.57^{Bb}	2.41^{Cb}
A. niger	-	2.84^{Ac}	1.95^{Cc}	1.72^{Ec}	2.07^{Bd}	1.82^{Dc}
C. albicans	-	2.48^{Ad}	1.86^{Cd}	1.69^{Ed}	2.26^{Bc}	1.77^{Dd}

2.84 (Log CFU/g). These finding are following the results of the antibacterial activity of free and encapsulated linseed and black seed oil mentioned before (free oils showed higher antibacterial activity than encapsulated form). This can be related to the reduction of bioactive properties of both linseed and black seed oils during emulsion preparation because of overheating caused by the ultrasound probe [63]. Moreover, the low concentration of antibacterial compounds released from alginate beads can be another reason for the lower antibacterial activity of encapsulated oil than free form. So, the oils cannot be diffused as well as free oil because are entrapped in the alginate bead and therefore, the amount of oil contacted to the bacteria will be lower compared to the free form [64]. In this regard, *Khalili et al.* (2015) [65] reported that encapsulated thyme essential oil was an effective antibacterial agent on tomato samples as it showed full preservation at a concentration of more than 700 (mg/L) for 5 days.

Different capital and small letters show significant differences in rows and columns, respectively ($p<0.05$). No treatment (control), without preservative inoculated with 105 (CFU/mL) microorganisms (GM), with 3% linseed oil inoculated with 105 (CFU/mL) microorganisms (GOM1), with 3% encapsulated linseed oil inoculated with 105 (CFU/mL) microorganisms (GEOM1), with 3% black seed oil inoculated with 105 (CFU/mL) microorganisms (GOM2), with 3% encapsulated black seed oil inoculated with 105 (CFU/mL) microorganisms (GEOM2)

Sensorial analysis

The sensorial properties of chocolate play a significant role in their consumer acceptability (*Ostrowska-Ligęza et al*. 2019). Fig. 5 show the sensorial properties of chocolate ganache treatments during 28 days of storage at 25°C. The incorporation of linseed and black seed oil in chocolate ganache led to a reduction in sensorial properties. In the other words, the control sample had the maximum appearance, odor, texture, and overall acceptability scores and the minimum odor and appearance scores were related to the GOM2 sample. Both GOM1 and GOM2 had the lowest texture and overall acceptability during storage time with no significant scores ($p>0.05$). Comparing the sensory scores of GOEM1 and GOEM2 with GOM1 and GOM2 samples showed that the encapsulation process could cover the undesirable sensory characteristics of the blended oils because GOEM1 and GOEM2 samples had higher sensory scores. In addition, both GOEM1 and GOEM2 samples did not differ significantly from the control sample. This can be attributed to the preservative and masking effect of encapsulation with alginate in oxidation stability and reduction of undesirable sensorial properties of applied oils. Generally, these results show the efficiency of encapsulation with alginate as natural compounds in food formulations without a high effect on their acceptability. In line with our study, *Perdones et al*. (2012) reported that coating strawberry with chitosan-lemon essential oils decreased the acceptability of samples in comparison to control [66]. Another study revealed that the incorporation of beads containing lavender and thyme oil in strawberry packaging system led to increasing the shelf life of samples with no undesirable effect on their sensorial properties [67].

No treatment (control), without preservative inoculated with 10^5 (CFU/mL) microorganisms (GM), with 3% linseed oil inoculated with 10^5 (CFU/mL) microorganisms (GOM1), with 3% encapsulated linseed oil inoculated with 10^5 (CFU/mL) microorganisms (GEOM1), with 3% black seed oil inoculated with 10^5 (CFU/mL) microorganisms (GOM2), with 3% encapsulated black seed oil inoculated with 10^5 (CFU/mL) microorganisms (GEOM2)

CONCLUSIONS

Linseed and black seed oil have various health-beneficial activities such as anticancer, antioxidant, and antibacterial activity, but their poor stability and bitter taste

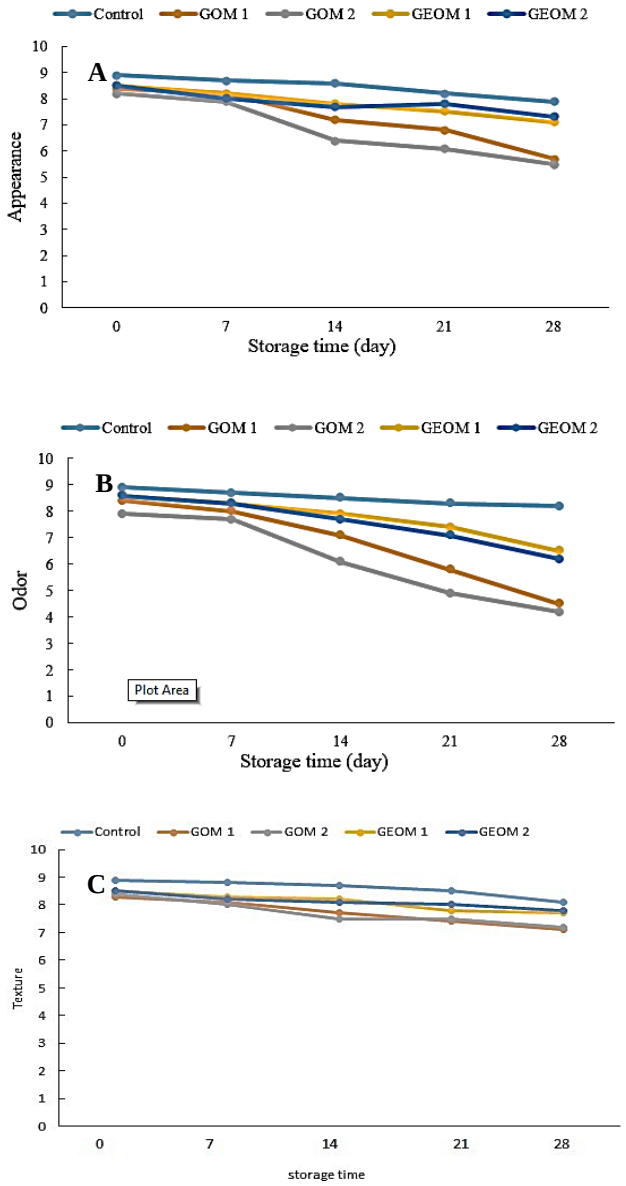

Fig. 5: Sensorial properties of chocolate ganache treatments during 28 days of storage at 25°C; A: Appearance, B: Odor, C: Texture

had led to their limited application in the food industry. Encapsulation with different carrier compounds can overcome these challenges. So, in this study, linseed and black seed oil were successfully loaded into alginate microcapsules. The evaluation of their antibacterial activity showed different inhibition potentials, as black seed oil had higher antibacterial activity, which can be attributed to their different composition. Both linseed and black seed oil in alginate bead showed lower antibacterial activity than free form either in culture media or in chocolate ganache.

The evaluation of sensorial properties of chocolate ganache samples revealed that samples containing encapsulated linseed or black seed oil had the lowest considerable differences to control samples. However, the free form showed higher antibacterial activity but in the regard to their protection from the undesirable condition, controlled release and marketability of product, loading the oils in alginate bead is a suitable way for application of black seed and linseed oil in food products. In conclusion, the hydrogel microcapsule produced in this study can be used as an effective carrier of bioactive compounds for application in food systems.

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