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Evaluation of Yoghurt Fortified with Encapsulated Echium Oil Rich in Stearidonic Acid as A Low-Fat Dairy Food

Hamdy A. Zahran[1]; Ahmed M. M. Mabrouk[2]; Heba H. Salama[2]*

[a]*Fats and Oils Department, Food Industries and Nutrition Research Division, National Research Centre, 12622 Dokki, Giza, Egypt.*

[b]*Dairy Department, Food Industries and Nutrition Research Division, National Research Centre, 12622 Dokki, Giza, Egypt.*

Abstract

Echium oil (EO) is oil rich in stearidonic acid (SDA) as well as linolenic acid, which have many health benefits, and protective roles in inflammation, cardiovascular disease (CVD) and cancer. The study aims to produce and evaluate yoghurt fortified with encapsulated echium oil (EEO) as a functional low-fat dairy food. The EEO was added to skimmed buffalo milk at rate of 0, 2, 4, and 6% (w/v) to create 4 treatments. Physiochemical, microbiological properties, and sensory acceptance were evaluated in the fresh samples and during the storage period at 5±2°C for 21 days. As the EEO increased, soluble nitrogen (WSN/TN ratio), acetaldehyde and viscosity increased while pH value decreased. In addition, the WSN/TN ratio, diacetyl, and viscosity gradually increased, but the pH value and acetaldehyde content decreased with increasing storage time. Yoghurt fortified with EEO had the lowest peroxide value compared to control sample. The addition of EEO had no significantly ($p \leq 0.05$) effect on viability of starter culture during storage period. The best of sensory evaluation was achieved by addition 2% EEO.

Keywords: Encapsulated echium oil; functional yoghurt; physicochemical composition

1. Introduction

Milk and dairy products, especially yoghurt, are an important contributor of human daily meal [1-4]. Yoghurt is one of the best products suitable for the delivery of ω-3 fatty acids, ensuring the utilization of their health benefits [5, 6].

Omega-3 fatty acids can be obtained from many sources, and it must be available in daily meals for good health and prevention of many diseases. Generally, dietary supplements and enriched foods are taken in for their fatty acid content, which come in sufficient quantities to meet the human daily dietary needs [7, 8]. The polyunsaturated fatty acids (PUFA), in particular, ω-3 fatty acids, have shown a triglyceride and cholesterol lowering effect [9-11]. The main reason for attracting stearidonic acid (SDA, 18:4, n-3) is that it is a more efficient endogenous conversion compared with that of *α*-linolenic acid (α-LA) [12]. Recent studies have shown the importance of vegetable oils rich in SDA and their healthy and protective role, which boils down to regulation of lipid metabolism, anti-inflammation and suppression of tumor survival [13-17]. The SDA affects insulin tropic and protects against diabetes, but this is still uncertain and under study [18, 13]. Oils containing SDA such as EO are a good food source for n3 fatty acids, which have a greater ability to increase EPA concentrations in tissues than those containing ALA-containing acids. Therefore, the use of EO in processing food products has been found to provide many nutritional alternatives to increase EPA concentrations in tissues [19, 20]. SDA can be used as a dietary supplement with chemotherapy for leukemia patients to enhance anti-tumor efficacy and chemotherapy drugs in dogs and possibly in humans with chemical-resistant lymphoma [21]. SDA have been shown to be more effective in compensating for immune stress [22]. SDA increased eicosapentaenoic acid (EPA) in red blood cells (RBC) [23, 24]. Glucose disposal was improved after EO consumption. The results suggested that PUFAs in

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EO supplementation have the capacity to alter circulating, RBC and muscle LC-PUFA levels and improve glucose tolerance in insulin-resistant monkeys [25]. SDA oil has therapeutic implications for several obesity-related pathologies [26]. On the other hand, a combination of phytosterols and ω-3 fatty acids has many health benefits towards lowering levels of cholesterol and triglyceride in the blood plasma and cardiovascular diseases in obese people [27-29,9].

Echium oil contains significant amounts of ω-3 fatty acids including 33% *α*-LA, 9-16% SDA, and 10% ω-6 fatty acid (γ-linolenic acid, γ-LA) [30]. The problem is this oil has low oxidation stability, which accelerates rancidity and results in undesirable primary and secondary oxidation products. This due to its highly content of polyunsaturated fatty acids [31]. To overcome this problem one can make use the encapsulation technology to protect echium oil from oxidative deterioration and preserve its quality [32-36, 29]. This study aims to evaluate the physiochemical, microbiological and sensory properties of functional low-fat yoghurt fortified with encapsulated echium oil, as a good source of ω-3 fatty acids, stearidonic acid, and phytosterols.

2. Materials and Methods

2.1. Materials

Fresh buffalo skim milk was procured from the Animal Production Research Institute, Agriculture Research Center, Giza, Egypt. Echium oil (EO) was supplied from De Wit Specialty Oils Co. (De Waal, Netherlands). Modified starch (EmCap) food grade and maltodextrin (C*Dry MD 01915) food grade were supplied from Cargill Co. (Istanbul, Turkey). *Streptococcus salivariu ssp. thermophiles* and *Lactobacillus delbrueckii sp. bulgaricus*, as starter cultures, were obtained from Chr. Hansen's Lab., A/S Copenhagen, Denmark. The starters were activated in sterilized skim milk (12%) for 24 h, to prepare mother culture.

2.2. Methods

2.2.1. Evaluation of quality properties of echium oil

Both acid and peroxide values of echium oil were determined according to the standard methods of AOAC [37].

2.2.2. Determination of fatty acid composition of echium oil

The fatty acid composition was determined by the transmethylation of the fatty chains to fatty acid methyl esters (FAMEs) according to the modified method by Zahran and Tawfeuk [38]. The FAMEs were separated with an HP 6890 plus gas chromatography (Hewlett Packard, USA), using a capillary column Supelco™ SP-2380 (60 m×0.25 mm×0.20 μm), (Sigma-Aldrich, USA), Detector (FID) and the injector and detector temperature was 250°C. The column temperature was 140°C (held for 5 min) and rose to 240°C, at rate of 4°C/min, and held at 240°C for 10 min. The carrier gas was helium at flow rate 1.2 mL min^{-1}. Sample volume was 1μL (in *n*-hexane) and injected through a split injector at splitting ratio of 100:20. FAMEs were identified by comparing their relative and absolute retention times to those authentic standards of FAMEs (Supelco™ 37component FAME mix). The fatty acid composition was reported as a relative percentage of the total peak area.

2.2.3. Microencapsulation by spray drying

2.2.3.1. Emulsion preparation

Maltodextrin and modified starch "EmCap" were mixed at a ratio of 9:1 (w/w), respectively. The hydrated solutions of wall materials (with concentrations of 30% "w/w on wet basis" and 70% distilled water), once prepared, were being stirred at room temperature (25˚C) for 2 h to attain a full hydration of the polymer particles [39]. The emulsion was prepared by blending EO (at concentration of 20% respect to the wall materials percent), then the emulsion was homogenized using a T18 digital ULTRA-TURRAX® homogenizer (IKA, Germany), at speed of 15 x 10^3 rpm/min for 5 min.

The spray drying process was performed in a laboratory scale spray dryer (Mini Spray Dryer B-290, BÜCHI Labortechnik AG, Flawil, Switzerland), with a nozzle atomization system with 1.5 mm and 100% aspirator capacity. The prepared emulsion was fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump rotation speed. Spray drying conditions were applied as follows: inlet temperature of 140°C, airflow rate at 0.439 m^3h^{-1}, pump rate at 4.5 mL min^{-1} [41].

2.2.3.2. Determination of particles size by Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) was conducted on a TESCAN microscope (TESCAN VEGA3^{TM}, Kohoutovice, Czech Republic) at an accelerating voltage of 20.00 kV and a working distance of 9.00 mm. Samples were sputter-coated with a gold-palladium mixture under vacuum prior to the examination. Particle diameters were measured from the SEM micrographs in their original magnification using the image software. Size distributions were obtained from a minimum of 200 measurements.

2.2.4. *Manufacture of functional yoghurt*

After buffalo's skim milk was heated to 90ºC/3 min, and cooled to 42ºC, EEO was added at rate of 0, 2, 4, and 6% (w/v). The milk mixtures were inoculated with 2% yoghurt starter (1:1) packed in 100 mL plastic cubs and incubated at 42°C until complete coagulation. The resultant functional low-fat yoghurt samples were stored at 5±2°C for 21 days [41]. Samples of functional yoghurt were analyzed for chemical, microbiological, sensory properties when fresh and during the storage period (21 days). All treatments were done two times during this study.

2.2.4.1. Chemical analysis

The moisture, nitrogen, fat and ash contents of the functional yoghurt were determined according to AOAC [35]. The protein content was obtained by multiplying the percentage of TN by 6.38. Diacetyl and acetaldehyde contents were determined according to Less and Jago [42].

2.2.4.2. Peroxide value

Lipids from the prepared yoghurt were extracted by chloroform/methanol (2:1, v/v) and filtrate using Whatman® Grade 1 filter paper, and the extraction was repeated three times. Evaporation of solvent was conducted at 50°C using rotary evaporator after extraction, leaving behind the lipid. The lipid was purged with a stream of nitrogen. Then the lipid was stored at ambient temperature in a screw-capped bottle wrapped with aluminum foil. The peroxide value determination was carried out spectrophotometrically according to the International IDF standard method 74A:1991 using a UV/VIS spectrophotometer (United Products & Instruments Inc., New Jersey, USA). Measurements were performed in triplicate. Hydroperoxide concentrations were determined using a Fe^{+3} standard curve with iron concentration varying from 1 to 24 µg, as described by Shantha and Decker [43].

2.2.4.3. Apparent viscosity

Apparent viscosity was measured at room temperature (25±1°C) using a Brookfield digital viscometer (Middleboro, MA 02346, U.S.A). The sample was subjected to shear rates ranging from 3 to 100 S^{-4} for an upward curve [44]. The viscosity was expressed as Pascal (Pa.s).

2.2.4.4. Microbiological analysis

Ten grams of yoghurt samples were homogenized with 90 mL of sterile physiological saline (0.85 % w/v NaCl); then the resulting homogenate was serially diluted up to 10^{-8} [45]. One milliliter from each dilution plated onto sterile petri dishes after that; M17 agar and de Mann-Rogosa-Sharpe (MRS) agar were poured for *Lb. delbrueckii* sub sp. *bulgaricuss* counts and *Str. Salivarius* sp. *thermophiles* counts respectively [46]. The plates were incubated at 37°C for 48 h under anaerobic condition for *Lb. delbrueckii* sub sp. *bulgaricuss* and aerobically at 37°C for 48 h for *Str. Salivarius* sp. *thermophilus*. Mold and yeast counts were determined by using potato dextrose agar acidified to pH 3.5 with sterile lactic acid (10%) according to APHA [47]. The plates were incubated at 25ºC for 3-5 days.

2.2.4.5. Sensory evaluation

Yoghurt fortified with or without different concentrations of EEO were evaluated, using sensory means when fresh (at zero time) and after 21 days of storage by ten panelists of the staff member of Dairy Department at Food Industries and Nutrition Division, National Research Centre, using the score sheet according to Badawi et al. [48].

2.3. Statistical analysis

All experiments and analyses were done in triplicate. The values of the means were statistically analyzed using SPSS software (version 22.0, 2013). The calculation comprised of analysis of variance one-way ANOVA and followed by Duncan test at significant level $p \leq 0.05$ according to Steel et al., [49].

3. Results and Discussion

3.1. Quality properties and fatty acid composition of echium oil

The free fatty acids can catalyst oxidative decay of oils by enzymatic and or chemical hydrolysis to form off volatile components [50]. Peroxide value (PV) is an indicator of peroxidation, and therefore the high peroxide value of the oil is a sign of a weak of oil resistance to oxidation during storage, and a hint to a deterioration level [51]. On this basis one might assume that the processed emulsions would be more stable than other highly unsaturated oils [52]. The acid and peroxide values of EO were 0.76 mg/g and 0.85 mEq./Kg oil, respectively (Table 1). These values were in acceptable levels set by the Codex Alimentarius Commission [53].

Table 1: Acid value, peroxide value and fatty acid composition of echium oil.

Item	Value ± SD
Acid value (mg/g)	0.76 ±0.03
Peroxide value (mEq. O_2/kg oil)	0.51 ±0.01
Fatty acids	(%)
Palmitic acid (16:0)	6.85 ±0.23
Stearic acid (18:0)	3.90 ±0.02
Oleic acid (18:1) n9, cis	15.41 ±0.07
Elaidic acid (18:1) n9, trans	0.71 ±0.01
Linoleic acid (18:2) n6	14.43 ±0.06
α-linolenic acid (18:3) n3	33.03 ±0.15
ɣ-linolenic acid (18:3) n6	10.41 ±0.05
Stearidonic acid (C18:4) n3	13.49 ±0.11
Arachidic acid (20:0)	0.89 ±0.06
Gondoic acid (20:1)	0.43 ±0.01
Behenic acid (22:0)	0.21 ±0.01
Others	0.27
SFA	11.84
UFA	87.89
ω-3 FAs	46.52
ω-6 FAs	24.83
ω-9 FAs	16.55

The fatty acids profile of EO was high in unsaturated fatty acids (UFA, 87.89%). The *α*-linolenic acid (18:3 n3) was the most abundant UFA in EO (33.03%), however, the percent of ω-3 fatty acids was the highest (46.52%) compared to ω-6 (24.83%) and ω-9 fatty acids (16.55%). The EO contains a unique combination of ω-3 and ω-6 fatty acids, which was represented in the α-linolenic acid (18:3, n3) and stearidonic acid (C18:4, n3) as ω-3, but linoleic acid (18:2, n6) and *ɣ*-linolenic acid (18:3, n6) were represented as ω-6 fatty acids.

The EO contains a unique combination of ω-3 and ω-6 fatty acids, represented in the α-linolenic acid (18:3, n3) and the stearidonic acid (C18:4, n3), however, linoleic acid (18:2, n6) and *ɣ*-linolenic acid (18:3, n6) were presented as ω-6 fatty acids. Additionally, oleic acid (18:1, n9) was presented as ω-9 fatty acids at a percentage of 15.41%, as well as a low amount (0.71%) of elaidic acid (18:1, n9, *trans*). Palmitic acid was present at the highest percentage (6.85%) of saturated fatty acids (SFA). These results are in agreement with those reported by Comunian et al. [29]; Guil-Guerrero et al. [54] and Mir [30].

3.2. Encapsulation characteristics

The using of wall materials such as modified starch "Em-Cap" and maltodextrin given a high microencapsulation efficiency (95.7%). Figure 1 shows the SEM external microstructures of powders produced by modified starch "Em-Cap" and maltodextrin. The results showed that size distribution of the encapsulated particles between about 0.5 to 10 µm. The particles characterized with a spherical shape and various sizes with no apparent cracks or gaps. These characteristics show that capsules have lower permeability to gases, increasing protection and retention of the active material. Furthermore, the variety in particle sizes is a typical distinguisher of particles produced by spray drying. Carneiro et al. [55] stated that microparticles morphology was influenced by different wall materials, which was compared with images from the combination of MD:Hi-Cap with the others, since this mixture resulted in microspheres with smoother surface and fewer teeth or coarseness.

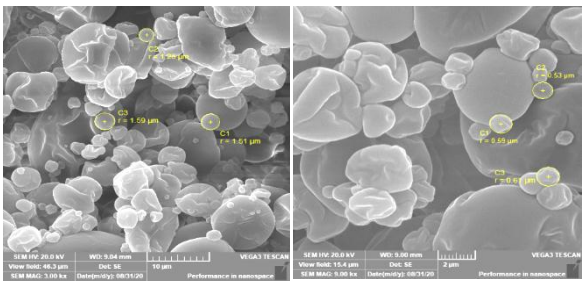


Fig. 1: Scanning electron microscope image of echium oil microcapsules

3.3. Chemical composition of Functional yoghurt

The composition of yoghurt fortified with different concentrations of EEO is represented in Table 2. The moisture content decreased, but protein, fat and ash contents increased as the addition of EEO increased. The moisture content significantly decreased as an increase in encapsulated oil, this is due to the increase of material percent used in the encapsulation process. This result is in agreement with Salama et al., [56] who found that the total solids increased and moisture content decreased as a function of encapsulated phenolic extract in liposome rate increased. On the other hand, protein and ash contents increased by increasing the ratio of added oil and this increase may be due to the materials used in the process of oil encapsulation. This increase in the fat percentage is due to the increase in the percentage of added encapsulated oil ranged from 2 to 6%. The chemical composition results were in line with Nagarajappa and Battula [57]; Salama et al., [44]; Salama et al., [56].

Table 2: Gross chemical composition of functional yoghurt fortified with different concentration of encapsulated echium oil

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	89.78^{D}	4.49^{A}	0.5^{A}	0.95^{A}
T1	88.35^{C}	4.66^{B}	0.9^{B}	1.03^{B}
T2	86.58^{B}	4.84^{C}	1.3^{C}	1.12^{C}
T3	84.92^{A}	4.94^{D}	1.7^{D}	1.22^{D}

*T1: yoghurt with 2% EEO; T2: yoghurt with 4% EEO; T3: yoghurt with 6% EEO. All treatments were done in triplicate. *Means with the different capital (A, B, C…) superscript letters within the same column indicate significant (P≤0.05) differences between treatments.*

The data of Table (3) shows that some chemical changes of yoghurt fortified with EEO during storage at 5±2°C for 21 days. The pH values significantly decreased by increasing the percentage of added EEO, which may indicate increased activity of the yoghurt starter used, this result was confirmed by the results of the microbiological analysis. During storage, the pH significantly decreased, reflecting increased acidity, as well as the decrease in pH value increased by increasing the excess of EEO.

The change in pH value during storage may be due to the continuous of starter activity, the nature of the ingredients used in the encapsulation process or the presence of some phenolic compounds that can bind to the protein and reduce the pH [44, 58, 56, 2]. Also, yoghurt starter was found to possess some antioxidant properties [59]; the proteolysis of milk protein [60] and production of organic acids [61] as ametabolic activity throughout the fermentation process and cold storage could be other sources of antioxidant activities [2]. The pH results agree with Comunian et al., [62] who reported that the incorporation of the microcapsules of EO, phytosterols and SA affected the pH and the titratable acidity of the yoghurt. Also, we confirmed that the pH around 4.0 is ideal for maintaining the structure of the microcapsules intact, preventing the release of the encapsulated materials during the storage of the yoghurt. Tamjidi et al., [63] and Serra et al., [64] explain that the decrease in pH and the increase in titratable acidity of the yoghurt samples during storage can be due to the metabolic activity of the starter used in yoghurt manufacture, which indicates the fermentation of lactose with the production of lactic acid.

Water Soluble nitrogen/Total Nitrogen Ratio (WSN/TN ratio) increased by increasing the percentage of EEO in functional yoghurt compared to control as well as storage because of the starter activity and hydrolysis of the protein. The addition of maltodextrin as an encapsulation material during encapsulation process of EO significantly enhanced the WSN/TN content in different yoghurt treatment and also storage period as confirmed by El Batawy and Khalil [65], whom reported that fortification of maltodextrin and storage period significantly influenced on the acidity, SN/TN ratio, diacetyle, acetaldehyde contents and viscosity in different yoghurt. The results are in line with Akl et al., [58]; Salama et al., [56] and confirmed by the microbiological examination presents in Table (4).

Acetaldehyde content was increased by increasing the rate of EEO addition to functional yoghurt from 2 - 6% to record the highest rate with T3 (6% contained EEO) and lowest ratio with control (without EEO) while its value decreased by storage to record the lowest values after 21 days of storage. This may be caused by the ability of lactic acid bacteria to convert acetaldehyde to ethanol, so it decreases during storage. Diacetyl also increased the excess of EEO compared to control, but in contrast to storage. This finding is consistent and confirmed by many researchers [66, 67, 68, 69, 56].

Table 3: Physicochemical analysis of functional yoghurt fortified with different concentration of encapsulated Echium oil.

Treatments	Storage (Days)	pH	(WSN/TN Ratio)	Acetaldehyde (µm/100g)	Diacetyl (µm/100g)
Control	0	5.52^{Cd}	12.03^{Aa}	11.66^{Ab}	3.81^{Aa}
T1		5.23^{Bd}	13.52^{Ba}	12.84^{Bc}	4.16^{Ba}
T2		5.17^{Bc}	13.43^{Ba}	16.68^{Cc}	4.51^{Ca}
T3		5.03^{Ac}	15.79^{Ca}	33.14^{Dd}	5.54^{Da}
Control	7	4.73^{Cc}	14.48^{Ab}	11.48^{Ab}	5.25^{Ab}
T1		4.68^{Bc}	14.38^{Aa}	11.58^{Ab}	7.10^{Bb}
T2		4.49^{Ab}	16.12^{Bb}	12.56^{Bb}	7.14^{Bb}
T3		4.48^{Ab}	16.60^{Ba}	15.62^{Cb}	7.14^{Bb}
Control	15	4.62^{Bb}	21.83^{Ac}	12.6^{Ac}	8.38^{Ac}
T1		4.61^{Bb}	22.10^{Ab}	15.5^{Bd}	8.58^{Ac}
T2		4.48^{Ab}	23.14^{Bc}	18.2^{Cd}	8.64^{Ac}
T3		4.46^{Ab}	24.90^{Cb}	22.4^{Dc}	13.24^{Bc}
Control	21	4.28^{Ba}	25.84^{Ad}	7.62^{Aa}	10.76^{Ac}
T1		4.43^{Da}	25.97^{Ac}	7.90^{Ba}	11.00^{Ad}
T2		4.34^{Ca}	25.83^{Ad}	8.06^{Ca}	12.80^{Bd}
T3		4.18^{Aa}	26.32^{Ab}	10.22^{Da}	16.84^{Cd}

*T1: yoghurt with 2% EEO; T2: yoghurt with 4% EEO; T3: yoghurt with 6% EEO.WSN/TN: Soluble nitrogen. All treatments were done in triplicate. *Means with the different capital (A, B, C…) superscript letters within the same column indicate significant (P≤0.05) differences between treatments. Means with the different small (a, b, c,) superscript letters within the same row are significantly (P≤0.05) different between storage period.*

Table 4: Viable counts (Log cfu/mL) of starters in yoghurt enriched with encapsulated echium oil during storage at 5±2°C for 21 days.

Strains	Time (days)	Control	T1	T2	T3
		Viable counts (log cfu/mL)			
Str. Salivarius spp. *thermophilus*	Zero	10.94^{Ac}	11.1^{Bc}	10.95^{Ac}	10.96^{Ad}
	7	10.91^{Bc}	11.0^{Bc}	11.1^{Bc}	10.4^{Ac}
	15	10.21^{Bb}	10.65^{Cb}	10.66^{Cb}	10.1^{Ab}
	21	9.28^{Aa}	10.23^{Da}	10.11^{Ca}	10.5^{Ba}
Lb. delbrueckii sub spp. *bulgaricus*	Zero	10.96^{Ad}	11.1^{Bb}	10.98^{ABc}	11.0^{ABd}
	7	10.93^{Bc}	11.13^{Cb}	11.1^{Cc}	10.52^{Ac}
	15	10.2^{Bb}	10.22^{Ba}	10.56^{Cb}	10.1^{Ab}
	21	9.57^{Aa}	10.13^{Ca}	10.2^{Ca}	9.7^{Ba}

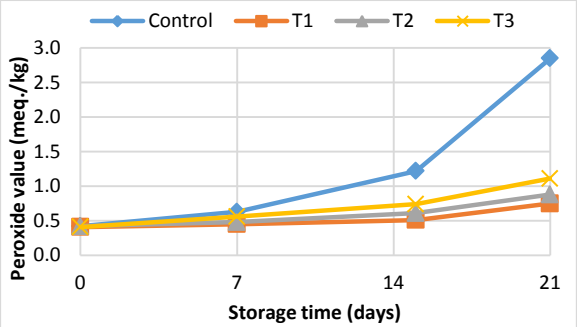
*T1: yoghurt with 2% EEO; T2: yoghurt with 4% EEO; T3: yoghurt with 6% EEO. All treatments were done in triplicate. *Means with the different capital (A, B, C…) superscript letters within the same column indicate significant (P≤0.05) differences between treatments. Means with the different small (a, b, c,) superscript letters within the same row are significantly (P≤0.05) different between treatment.*

3.4. Oxidative stability of functional yoghurt during storage

The peroxide value was used to measure the oxidative stability of yoghurt samples fortified with EEO compared to control sample (Fig. 2). All samples, at zero time, exhibited a low level of oxidation, fluctuating from 0.41 to 0.42 mEq/kg oil. After one-week of storage, there is no significant variation between all samples and they did not differ from each other; whereas, after two weeks of storage, we found changes in peroxide values, reaching values of 0.51, 0.61and 0.74mEq/kg oil for yoghurt samples treated by 2, 4 and 6% encapsulated EO, respectively.

On the other hand, the peroxide value of control sample reached to 1.22 mEq/kg oil at same time and to 2.85 mEq/kg oil after three weeks of storage. However, samples treated with encapsulated EO suffered a significant increase in peroxides at the third week of storage, in order of 2 < 4 < 6% of encapsulated oil contained. The oxidative stability of prepared yoghurt was powerfully influenced by increasing the ratio of the encapsulated EO, due to the existence of adhering non-encapsulated oil around the particles of powder. The encapsulation of oils contains highly polyunsaturated fatty acids affecting the oxidative stability of food matrix. Soliman et al., [70] applied the encapsulated wheat germ oil (WGO) in preparing functional labneh cheese; they also studied the oxidative stability using peroxide value. The authors found that the oxidative stability was enhanced by using encapsulated WGO as compared to free oil as a control sample during the exposure to UV radiation.

Fig. (2): Changes in peroxide values of yoghurt samples enriched with encapsulated echium oi during storage at 5 ±1℃ for 21 days.



3.5. Apparent viscosity

Fig. 3 presents the viscosity (Pa.s) of yoghurt fortified with different concentrations of EEO fresh and up to 21 days of cold storage. The viscosity of functional low-fat yoghurt significantly increased with the increase in EEO and storage time. Moreover, maltodextrin used in the capsule process plays a role in binding free water, which increases the viscosity of functional low-fat yoghurt. The results of the viscosity in the fresh yoghurt are consistent with that found by Salama et al. [54]. Increased viscosity with storage time is due to increased ability to bind to water; the particles are more swollen, and are connected to each other over a larger area [71]. In addition, the bonds between the particles of the gel became stronger or their numbers are greater. The reason for the increase in viscosity during storage is due to the increase in total solids (Tab. 3), which have increased in storage and the presence of maltodextrin, this emulsifies and water binds to yoghurt. Viscosity results agree with Comunian et al., [62] who decided the increase in viscosity is due to the addition of the microcapsules, which increases the solids content of the resulted yoghurt.

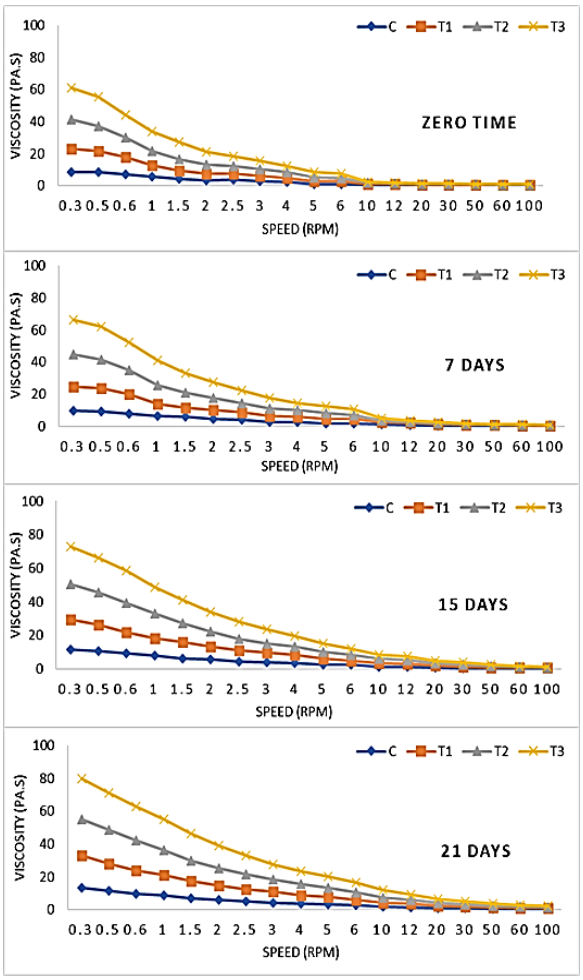


Fig. (3): Viscosity of functional yoghurt fortified with different concentration of encapsulated echium oil during fresh and during storage up to 21 days.

3.6. Microbiological analysis

The data presented in Table 4 showed the effects of EEO on the viable counts of yoghurt starters during storage at 5±2°C for 21 days. At zero time, the viable counts of yoghurt starters were recorded high viable counts in all treatments. The viable counts of *Str. Salivarius*sp. *thermophilus* reached to 10.94-log cfu/g in control treatment and

then gradually decreased by one log cycle and reached to 9.28-log cfu/g at the end of storage period. In addition, the viable counts of *Lb. delbrueckii* sub sp. *bulgaricus* in the control treatment decreased from 10.96 to 9.57-log cfu/g at zero time and after 21 days of storage time respectively. On the other hand, the treatment supplemented by 2% EEO, the counts of both *Str. Salivarius*sp. *thermophilus* and *Lb. delbrueckii* sub sp. *bulgaricus* were 11.10-log cfu/g at zero time and decreased to 10.23 and 10.13-log cfu/g with them respectively. The viable counts in treatments supplemented by 4% are still in the same log cycle without any changes. By increasing the level of EEO to 6%, the viable counts of starters are still above 10.0-log cfu/g in all treatments except *Lb. delbrueckii* sub sp. *Bulgaricus;* it decreased to 9.7-log cfu/g at the end of the storage period. Our results were in agreement with those obtained by Bello et al., [72] who reported that the addition of EO to the milk for producing functional yoghurt enhances the growth and viable counts of the starter cultures and the counts of *Streptococcus* and *Lactobacillus* were remained about 10^8 cfu/g and 10^7 cfu/g respectively. Form the obtained results coliform bacteria was not detected in both control and treatments when fresh or during storage time which reflect the good hygienic conditions during the manufacture of functional yoghurt and storage. For that, the yoghurt was safe for consumption.

3.7. Sensory evaluation

The data presented in Table 5 shows that the addition of EEO in different concentrations in this study did not affect the color of fresh and storage yoghurt. The observation in color agrees with Nagarajappa and Battula [57] who provided evidence that there was no significant difference in color between control and milk fortified with encapsulated flaxseed oil, phytosterols and polydextrose. The addition of 2% (T1) of the EEO showed approval and was accepted by the panelists in the different tested parameters (taste, aroma, and mouth-feel) as well as the overall acceptability followed by the control, then T2 and then T3. This result was in agreement with Nagarajappa and Battula [57]; Lim et al. [73] who explained that this may be due to the higher level of oil and phytosterols in yoghurt. The favorable treatment selected by panelists was T1 and that is the study recommended according to the acceptability of consumers. The choice of 2% (T1) is the best according to sensory evaluation and is economically preferable. The yoghurt with EEO offered good sensorial acceptance. It was conceivable to apply the EEO in yoghurt as recommended by Comunian et al., [62].

Table 5: Sensory evaluation of functional yoghurt fortified with different concentration of encapsulated echium oil.

*All treatments were done in triplicate. *Means with the different capital (A, B, C…) superscript letters within the same column indicate significant (P≤0.05) differences between treatments. *Means with the different small (a, b, c,) superscript letters within the same row are significantly (P≤0.05) different between treatment.*

Parameters	Storage Period (days)	Control	T1	T2	T3
Color	Fresh	8.0^{Aa}	8.0^{Aa}	8.0^{Aa}	8.0^{Aa}
	21	8.0^{Aa}	8.0^{Aa}	8.0^{Aa}	8.0^{Aa}
Taste	Fresh	7.0^{Aa}	7.5^{Az}	6.5^{Aa}	5.0^{Aa}
	21	8.0^{Ab}	8.0^{Ab}	7.0^{Ab}	6.0^{Ab}
Aroma	Fresh	7.0^{Aa}	7.5^{Aa}	6.0^{Aa}	5.5^{Aa}
	21	7.5^{Ab}	8.0^{Ab}	6.3^{Ab}	6.0^{Ab}
Mouth-feel	Fresh	7.0^{Aa}	7.5^{Aa}	6.0^{Aa}	5.0^{Aa}
	21	7.4^{Ab}	7.9^{Ab}	6.4^{Ab}	5.7^{Ab}
Overall Acceptability	Fresh	7.0^{ABa}	8.0^{Ba}	6.3^{ABa}	5.0^{Aa}
	21	7.5^{ABb}	8.6^{Bb}	6.5^{ABb}	5.4^{Ab}

4. Conclusion

Echium oil encapsulation is an effective method to maintain active compounds by adding them to various food products. Yoghurt is a good delivery system for encapsulated oils and for many active components. The addition of EEO has significant effects on the physicochemical properties of yoghurt, and had no negative effects on viable count of starters during storage. Encapsulation technology of oxidative and sensitive oils rich in ω-3 fatty acids are one of the most promising ways to increase fat stability and protect bioactive components, which can easily add to various food products, such as low-fat dairy products. The fortification of dairy products by bioactive agents, in particular oils rich in omega fatty acids, still needs more further studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

There is no Informed consent.

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