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Formulation of Multi-Functional Omega-3 Oil Rich Microcapsules by Spray Drying Methodology

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Abstract

This work was proposed to prepare multi-functional omega-3 oil based microcapsules. Rosemary (*Rosmarinus officianlis* L.) extracts as potential active compounds were used to stabilize these omega-3 rich microcapsules against both oxidation and microbes. Solvents of different polarities as well as ultrasonic-assisted water extraction (UAE) technique were tested for preparing promising extracts. Results demonstrated that water extract showed the highest phenolic extract as well as highest radical scavenging activity (RSA %) even when compared with BHT as a reference synthetic antioxidant. However, the potency of ultrasonic-assisted water extract as a radical scavenger was greatly enhanced specifically at a temperature of 45°C and 30 min extraction time. Regarding the antimicrobial activity, although giving highest total phenolics and RSA %, water extract had no antimicrobial activity against both Gram negative or Gram positive bacteria (at concentration range 0.25 –15%) while methanol extract revealed inhibitory activity against all tested pathogens at concentration of 5% or higher. Moreover, methanol extract didn't affect the survivability of the beneficial bacteria of lactobacilli strains. Finally, according to the results the microcapsules prepared by spray drying method exhibited better protection of omega-3 rich oils and also the yogurt fortified with spray-dried fish oil capsules had higher score in sensory evaluation.

Key words: Antimicrobial activity, Antioxidant activity, Omeg-3 rich oils, Rosemary extract, Ultrasound-assisted extraction.

1. Introduction

Though lipids constitute only small amount of many foods, they contribute significantly to its flavor, odor, color, and texture as well as sharing in palatability, satiety and being a very important source of energy.Omega-3 polyunsaturated fatty acids represent a group of fatty acids including α-linolenicacid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The metabolism of α-linolenic acid in humans has been well characterized. After ingestion of α-linolenic acid-rich oils there was an increase in plasma and tissue concentrations of very-long-chain polyunsaturated fatty acids eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n−3) [1]. A major consequence of α-linolenic acid deficiency is that its chief end products, EPA and DHA, are not adequately produced [2]. Because docosahexaenoic acid is a major component of the phospholipid membranes of the brain and retina, its deficiency in these organs then leads to abnormal function [3]. The beneficial health effects of fish oil which is the richest source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are greatly observed in the Greenland Eskimos who consumed a high seafood diet and had low rates of coronary heart disease, asthma, type 1 diabetes mellitus, and multiple sclerosis [4]. Omega−3 fatty acid deficiency is induced when there is simultaneously a high

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content of linoleic (C18:2) acid in the diet, which tends to inhibit the synthesis of docosahexaenoic acid from linolenic (C18:3) acid [5]. Thus, diets rich in corn, safflower, sunflower, and peanut oils, all of which are high in linoleic acid and low in α-linolenic acid, can lead to Omega-3 fatty acid deficiency. Thus, a high ratio of Omega-6 to Omega-3 fatty acids in the diet induced Omega−3 fatty acid deficiency [6].

Hence, it is necessary to increase omega-3 fatty acids (ALA, EPA, or DHA) concentration in our diet or increase uptake of omega-3 rich oils (flaxseed or fish oils). However, the main problem lies on the ready susceptibility of these omega-3 oils to oxidation which is reflected in quick deterioration during storage or food processing and short shelf-life [7-9]. Many attempts have been made to prevent the oxidative deterioration of lipids in general and omega-3 oils in particular by using natural antioxidants [10-14]. Labiatae family (i.e. rosemary, sage, and thyme) and especially rosemary (*Rosmarinus officinalis* L.) revealed strong antioxidant activity in stabilizing polyunsaturated oils [11]. Another approach to preserve and protect lipids from oxidation is through microencapsulation, which has been widely used in manufacturing powder-type oil and fat products [16-17].

The objective of this work was to combine the effectiveness of both natural antioxidants and microencapsulation to prepare multi-functional omega-3 rich microcapsules that could be used in fortification of yogurt as a representative food candidate or as is in pharmaceutical formulations.

2. Materials and Methods

2.1. Materials

2.1.1. Oils and chemicals

Flaxseeds (*Linum usitatissimum* L.) were bought from the Agricultural Research Center, Giza, Egypt. Rosemary (*Rosmarinus officinalis* L.) leaves were bought from local herb stores in Cairo, Egypt. Fish oil gelatin capsules (Cod-liver oil) were bought from a pharmacy in Dokki District, Giza, Egypt. α-Tocopherol (97%) and Folin-Ciocalteu reagent, 2,2-Dipheny l-1- picryl hydrazyl (DPPH), Butyl hydroxyl toluene (BHT), were purchased from Sigma-Aldrich Chemical Co., (St. Louis, USA). Gum Arabic was obtained from Edwic Chemical Company, Cairo, Egypt. Maltodextrin (DE 15) from PPZ "NOWAMYL" S.A. (Lobez, Poland). All the solvents and other chemicals used were of analytical grade.

2.1.2. Microbial strains for antimicrobial assay

Bacillus cereus B-3711 (Gram positive) and *Aspergillusflavus* 3357 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL). *Listeria monocytogenes* (Gram negative) 598 was provided by the Department of Food Science, University of Massashusetts, Ambert MA, USA. *Escherichia coli* 0157: H7 (Gram negative) and *Staphylococcus aureus* (Gram positive) were isolated and serologically identified by dairy microbiological Lab., National Research Centre. *Yersinia enterocolitica* (Gram negative) was obtained from Hungarian National Collection of Medical Bacteria, OKI, Gyaliut 2-6, H-1966 Budapest, Hungary. *Pseudomonas aeruginosa* (Gram negative) and *Aspergillusniger* were obtained from Department of Microbiology, Swedish University of Agricultural Sciences. *Salmonella typhamirum* (Gram negative) was provided from Hungarian National Collection of Medical Bacteria, OKI, Gyaliut 2-6, H-1966 Budapest, Hungary.

2.1.3. Lactobacilli strains

Lactobacillus helveticus CNRZ 32 was obtained from Dairy Microbiological Lab., Centre de recherché eszoo techniques (Jouy-en-Josas, France, *Lactobacillus acidophilus* CH-2 and *Lactobacillus reuteri* ch-1 were obtained from Chr. Hansen's Lab., Denmark. *Lactobacillus rhamnosus* 306 and *Lactobacillus plantarum*4496 were provided by Northern Regional Research Laboratory (NRRL), *Lactobacillus casei* FEGY 9973was obtained from Faculty of Agriculture, Cairo University, Egypt.

2.1.4. Starter cultures for yogurt preparation

Commercial starter *cultures Lactobacillus dulbrueckii* sub spp. *bulgaricus* Lb-12 DRI-VAC and *Streptococcus thermophilus* CH-1 obtained from Chr. Hansen's Lab., Denmark, were used for manufacture of yogurt.

2.2. Methods

2.2.1. Extraction of flaxseed oil

Flaxseed oil was extracted from the corresponding seeds by using hydraulic pressing at room temperature as described by Ustun *et al.* [18], and modified by Hamed *et al.* [19]. Briefly, the dry seeds were milled into a fine powder, then batches of the powder, 250 g each, were wrapped in a thick heavy-duty cloth and the oil was extracted using an in-house made hydraulic laboratory scale pressing machine manufactured at the National Research Centre, Cairo, Egypt and operated at its maximum pressure (3500 psi) for 1.0 h at room temperature. The crude oil was filtered through a thin cotton cloth. The extracted flaxseed oil was stored in dark bottles at -20°C, while the purchased fish oil gel capsules were stored at 4°C (as recommended by the manufacturing company), until being used for following analyses.

2.2.2. Chemical characteristics of oils

Peroxide value (PV), acid value (AV), iodine value (IV), and Saponification (SV) of tested oils were carried out according to American Oil Chemists' Society [20]. All evaluations were performed in triplicate.

2.2.3. Fatty acid composition

Determination of fatty acids composition was carried out according to our previous works [21, 22] using a Hewlett Packard HP 6890 gas chromatograph, operated under the following conditions: Detector, flame ionization (FID); column, capillary, 30.0 m X 530 µ m, 1.0 µ m thickness, polyethylene glycol phase (INNO Wax); N_2 with flow rate, 15 mL/min with average velocity 89 cm/s (8.2 psi); H2 flow rate, 30 ml/min; air flow rate, 300 ml/min; split ratio, 8:1, split flow, 120 ml/min; gas saver, 20 ml/min. Detector temperature, 280°C; column temperature, 240°C; injection temperature, 280°C. Programmed temperature starting from 100°C to reach a maximum of 240°C was used for eluting the fatty acid methyl esters. The identification of the peaks was made as compared with chromatograms of standard fatty acids methyl esters (Sigma, USA).

2.2.4. Traditional solvent extraction of rosemary phenolic compounds

Extraction was carried out according to Hamed and Abo-Elwafa [10]. Briefly, two grams of dried ground sample were mixed with 20 mL (1:10 solid to solvent ratio) of three solvents of different polarity namely hexane, methanol and distilled water in a beaker and placed in an ultrasonic bath (Bandelin Sonorex, Germany, 480 W, 35 KHZ) for 20 min. The extract was then filtered (Whatman No. 1). The extraction process was repeated two times more and the filtrates were combined. The combined filtrate of each extract was then dried using rotary evaporator under reduced pressure. The dry extract obtained with each solvent was weighed to determine the yield percentage and kept at -20°C until tested. The extract yields were calculated as follows:

$$Yield\ (\%) = [Ext/S] * 100$$

Where, Ext: weight of the extract and S: weight of the initial powdered plant.

Before use in total phenol determination or radical scavenging activity, the hexane extract was dissolved in 10 mL dimethyl sulfoxide (DMSO), while methanol and aqueous extracts were dissolved in 10 mL methanol.

Note: Solvent giving the best results depending on yield %, total phenolic content (TPC), and radical scavenging activity (RSA %) was taken as the best solvent for following experiments.

2.2.5. Ultrasonic-assisted extraction

Ultrasonic Homogenizer (Omni Sonic Ruptor 400 Ultrasonic Homogenizer, Kennesaw, USA) with a standard probe diameter of 2.54 cm was used for leaching of phenolic compounds. It has a maximum power of 300 W and 20 kHz frequency. The power level chosen, 70%. Ultrasound was operated at 50% pulser mode. Extraction temperatures were25±1°C and 45±1°C using water bath. Solid to solvent ratio was kept at 1:10. Ten g of sample was placed into a 600 mL beaker with the appropriate amount of distilled water. The beaker was placed into cooled water bath and ultrasonic probe

was dipped at most 1.5 cm depth into the extraction media. Extractions were performed at different times (10, 20, and 30 min).

2.2.6. Total phenolics in plant extracts

The total phenolic compounds in the different extracts were determined colorimetrically at 725 nm, using the Folin-Ciocalteau assay [23]. Calculations of the total phenolic content (expressed as gallic acid equivalent, µg GAE/g extract) were done using a standard curve of different concentrations of gallic acid ranging from 0.0 to 450.0 mg/mL. All of the samples were run in triplicate.

2.2.7. DPPH free radical scavenging assay

The free radical scavenging activity (RSA %) of the extracts was measured using DPPH by the method of Blois [24]. In brief, a 0.1 mM solution of DPPH in methanol was prepared and 2 ml of this solution was added to 200 µl (100 ppm) of each extract solution to be tested. After 30 min, absorbance was measured at 517 nm. Two controls for this test, a negative control (containing all reagents except the test sample) and 100 ppm of butylated hydroxyl toluene (BHT) as a positive control). The scavenging activity was calculated as follows:

$$RSA\ \% = [(A_{control} - A_{sample} / A_{control}] * 100$$

Where $A_{control}$ is the absorbance without extract and A_{sample} is the absorbance with the extract.

2.2.8. Determination of tocopherol content by HPLC

The analysis of tocopherol content was performed with an HPLC method [25]. Briefly, the analysis was executed at room temperature on a Waters 600 high performance liquid chromatograph equipped with a Symmetry C18 column (150 mm×3.9 mm, 5 µm) fitted with a µ Bondapak C cartridge guard column (all from Waters, Millford, Ma, USA). Mobile phase of acetonitrile and methanol (1:1, v/v) was used at the flow rate of 1 mL/min. Samples of oils were weighted (0.12 g) and dissolved in 1 mL of 2-propanol. Vortex-mixed samples were directly injected onto the HPLC column without any additional sample treatment. Injection volume was 20 µL. The eluate was detected using a Waters 474 scanning fluorescence detector set for the emission at 325 nm and excitation at 295 nm. Tocopherols were identified by comparing their retention times with those of the corresponding standards.

2.2.9. Assessment of the antimicrobial activity of rosemary extract against pathogenic strains

The antimicrobial activity was assessed by the agar–well diffusion method [26]. Firstly, 1.5 g of each rosemary extract (i.e. water or methanol extract) was dissolved in 10 mL DMSO (dimethyl sulfoxide) to get 15% solution, after that different dilutions were prepared in DMSO to get 0.25, 0.5, 0.75, 1, 3, 5, 7, 10 and 15% solutions. Then the inoculum suspension of each activated pathogenic strain was adjusted to a suspension of 10^5cells. The inoculums suspension was spread over a nutrient agar plate, to achieve confluent growth, and allowed to dry. 10 mm- diameter wells were bored in the agar using a sterile cork borer and the agar discs were removed. A 100 µl of the each extract concentration was placed into a well with micropipette and the plate was incubated for 24 h at 37ºC. After incubation, the diameters of the zones of inhibition were measured to the nearest mm.

2.2.10. Effect of different rosemary extracts on Lactobacilli species

To determine the effect of rosemary extracts on lactobacilli, different strains of lactobacilli (*L. helveticus, L. acidophilus, L. rhamnosus, L. reuteri, L. casei*and *L. plantarum*) were activated individually and grown in MRS broth medium at 37ºC using 2% inoculums. The molted sterilized MRS agar medium was fortified with 3, 5, 7 and 10.0% (v/v) of extract individually. 1ml of each activated strain was added in petri dish approximately 10^5 colony forming units (CFU/mL) and the 15 mL molted MRS medium contained different concentrations of extract added to each plate, and then mixed the inoculums with the agar medium. The plates were solidified and incubated at 37ºC for 48 nh under anaerobic condition [27].

2.2.11. Microencapsulation techniques

2.2.11.1. Emulsion formulation

An emulsion of omega-3 rich (fish or flaxseed) oil-in-water was formulated as an initial step for microencapsulation using spray drying. The emulsion was formed by 15 g of gum Arabic (GA) was hydrated in 100 mL deionized water to prepare. This solution was left overnight at 4ºC to allow full hydration. Then, 15.0 g of maltodextrin (MD) was dissolved in the same gum solution to reach a ratio of 1:1 GA/MD, w/w). Tween 80 (1.0% w/v) was dissolved in the GA/MD solution at intensive mixing followed by the addition of certain weight of the omega-3 oil to obtain a 10% w/v emulsion. The whole mixture was magnetically stirred for 5.0 minutes to form a coarse pre-emulsion then was homogenized for 20 minutes at full sonicator power (200.0 Watt, 24kHz) using UP200S ultrasound homogenizer (IKA Hielscher GmbH, Berlin, Germany) equipped with an impulse generator 14 mm in diameter. To avoid the rise of emulsion temperature above ambient during ultrasound homogenization, the emulsion vessel was immersed in a cold water bath during the whole process [28].

2.2.11.2. Spray-drying technique

A co-current Mini Spray Dryer B-290 (BÜCHI, Flawil, Switzerland) was used. The dimensions of the glass dryer chamber were 0.45 m in height and 0.14 m in diameter. The spraying system consisted of a two-fluid nozzle composed of an internal tip with an opening 0.7 mm in diameter and an external ring with an opening of 1.5 mm in diameter. The constant process parameters included drying air flow rate 85.0% of suction fan controller. The inlet and outlet temperatures were 160.0°C and 80.0°C ±1.0°C, respectively. The resulted powder was collected from both the drying chamber wall and from the cyclone. The drying process was conducted twice for two different batches of each emulsion.

2.2.11.3. Extrusion technique

The encapsulating materials were prepared as follows Sodium alginate aqueous solution (3%) was first prepared in distilled water. Arabic gum was dissolved in water in the concentration 0.5%. The microencapsulation process was performed using the extrusion technique [29]. One part of each oil (fish or flaxseed) was mixed separately with nine parts of the prepared sodium alginate (3%) and rosemary extract (5%) with gentle stirring for 10 min to make mixture fortified with 10% oil and added 0.02% tween 80. The mixture was then extruded into the hardening solution ($CaCl_2$, 0.2 M) through sterile syringe (0.5 mm) with gentle stirring for 30 min. Then after extruding the alginate-oil mixture into the hardening solution, the harvested alginate microcapsules were then coated with Arabic gum by immersing (about 15 g capsules) in 100 ml of Arabic gum solutions with gentle stirring for 30 min. The formed microcapsules were harvested by filtration then washed by sterile saline solution and dried in the room temperature for 24h, next day the microcapsules were stored refrigerator till be used.

2.2.12. Encapsulation efficiency

Encapsulation efficiency (EE) was determined according to the method mentioned by Hashim *et al.* [30] with some modification. Fifty milliliters of n-hexane were added to 3 g of micro-beads in 100 ml beaker followed by shaking for 2 min at room temperature for the extraction of surface oil. The mixture was filtered through a Whatman filter paper no. 1, and the micro-beads collected on the filter were rinsed three times with 20 mL of hexane. Then, the solvent was evaporated at reduced pressure until constant weight. The non-encapsulated oil (surface oil) was determined by mass difference between the initial clean flask and that containing the extracted oil residue. Total oil was assumed to be equal to the initial oil, since preliminary tests revealed that all the initial oil was retained, which was expected, since both flaxseed and fish oils are non-volatile. EE were calculated as follow:

$$EE\ \% = [(TO-SO)/TO]*100$$

Where TO and SO are total oil and surface oil, respectively.

2.2.13. Antimicrobial effect of microcapsules

Bacterial inhibition in growth medium was studied using time-kill assays according to Davidson

and Parish [31] with some modification. Ten mL oftryptone soya broth medium was prepared in test tubes and sterilized by autoclave at 121ºC for 15 min. The test tubes inoculated individually with pathogenic strains (~X10^3). One gm of each microcapsule type was added to each tube and incubated for 24 h at 37ºC. A negative control group of test tubes without microcapsules was also inoculated individually with pathogenic strains. Moreover, another group of test tubes containing free oil (without capsulation) was individually inoculated with pathogenic strains and free type of oil as positive control group. A bacterial suspension sample (1.0 mL) was serially diluted in 9 mL saline solution, plated in 1.0 mL using nutrient agar, incubated for 24 h at the optimum growth temperature, and then enumerated for viable count as CFU/mL.

2.2.14. Production of yogurt using microcapsules

Fresh buffalo`s milk standardized to 5% fat, heated at 72ºC for 15 min then cooled and adjusted to 42ºC according to Abd EL-Khalek *et al.,* [32], The milk was divided into five portions and starters were added as following:

1. The first portion inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and was regarded as control.
2. The second portion was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% fish oil encapsulated with extrusion method
3. The third portion was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% fish oil encapsulated with spray drying method
4. The fourth was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% flaxseed oil encapsulated with extrusion method
5. The fifth portion was inoculated with (1:1%) *St. thermophilus* and *Lb. bulgaricus* and 1% flaxseed oil encapsulated with spry drier method

Then, samples were transferred into 40 ml plastic cups and incubated at 42ºC for 2 to 4 h until coagulation and stored at 7 ºC for 15 days. The pH of the final yogurt reached to 4.60. The produced yogurt treatments were evaluated for its microbiological activity and organoleptic properties.

2.2.15. Lactobacilli counts

Lactobacilli counts were determined using MRS agar according to IDF, [33]. The plates were incubated at 37°C for 48 h under anaerobic condition.

2.2.16. Streptococci counts

Streptococci counts were determined using M17 agar according to IDF, [33]. The plates were incubated at 37°C for 48 h.

2.2.17. Organoleptic assessment

The Organoleptic properties of yogurt samples were assessed by a regular taste panel of the staff- members of the dairy science department, National Research Center. Yogurt samples were evaluated for flavor (50 points), body and texture (40 points) and appearance (10 points) according to Bodyfelt *et al.* [34].

2.2.18. Oxidative stability of encapsulated oils

Oxidative stability of encapsulated oils were followed up by measuring the conjugated diene, conjugated triene (primary oxidation products) and *p*-anisidine value (secondary oxidation product) as described in Official methods and recommended practices of the AOCS [20].

2.3. Statistical analysis

All the data were expressed as mean ±standard error of the mean (SEM) of three independent assays and analyzed through unpaired Student's t-test or ANOVA combined with Tukey's test (Graph Pad Prism 5). P values of less than 5% ($p < 0.05$), were considered to be significant.

3. Results

3.1. Chemical characteristics of oils

Table 1 shows the oil content and the chemical characteristics of flaxseed and fish oils. The

oil content of cold pressed flaxseeds was 21.3% which was in accordance to that reported by Ogunronbi *et al.* [35] who found that the oil content obtained from four different batches of flaxseeds were in the range 12.4 - 22.5%. The high residual oil content in the meal was therefore not surprising as the cold-press process is a less thorough oil extraction process than the solvent extraction process usually employed for oil meals. According to the Codex Alimentarius Standard [36] for edible fats and oils cold pressed oils should have acid value less than 4.0 mg KOH/g oil and peroxide value less than 15 mEq O_2/kg oil. Regarding fish oil, according to Codex Alimentarius Standard PV should be less than 5 mEq O_2/kg oil and AV less than 3 mg KOH/g oil.

As shown in Table 1. PV of 1.4 and 3.19 and AV of 0.26 and 0.99 mg/g for flaxseed and fish oils, respectively, were a result of lower hydrolysis of triglycerides and signified that these oils could have a long shelf life (under good storage conditions), which allows it to be consumed as edible oil. The high IV for flaxseed oil (139.13 g/100g) and for fish oil (150.2 g/100g) as presented in Table 1 were a result of their high content of unsaturated fatty acids as would be seen in their fatty acids (Table 2). Also, as shown in Table 1.the presence of considerable amounts of tocopherols in flaxseed oil (434 μg/g oil) and in fish oil (167 μg/g oil) enhance partly their good quality.

Table 1
Chemical characteristics of flaxseed and fish oils

Parameter	Flaxseed oil	Fish oil
Oil yield (%)	21.3 ±0.46*	(-)
Peroxide value (meq.O_2/kg oil)	1.4 ±0.12	3.19 ±0.18
Acid value (mg KOH/g oil)	0.26 ±0.04	0.99 ±0.08
Iodine value (g Iodine /kg oil)	139.13 ±1.03	150.2 ±1.89
Saponification value (mg KOH/g oil)	197.58 ±2.50	181.95 ±3.90
Tocopherol (μg/g oil)	434 ±5.50	167 ±2.65

* Each value represents mean ± SD (n = 3)

3.2. Fatty acid composition

The fatty acid profile of the oil extracted from the flaxseeds as well as that of the purchased fish oil are presented in Table 2. Regarding flaxseed oil α-linolenic acid (ALA/C18:3, omega-3 fatty acid), a polyunsaturated fatty acid, was the predominant fatty acid (62.68%) followed by oleic acid (C18:1, omega-9 fatty acid) and linoleic acid (C18:2, omega-6 fatty acid) constituting 14.44% and 14.27%, respectively. The high ALA content is an indication of the degree of unsaturation of flaxseed oil [37]. In accordance of our results several workers have reported values in the range of 51.1 to 64.6% for ALA and in the range 12.5 to 18.9 for oleic acid and linoleic acid, respectively, in flaxseed oil [37-39]. Given the appreciable amounts of ALA, flaxseed oil could be a good source of omega-3 fatty acids if added to conventional foods, depending on the percentage of inclusion in such foods.

As shown in Table 2 fish oil was characterized by the presence of considerable amount of eicosapentaenoic acid (EPA, C20:5, omega-3 fatty acid) representing about 12% and docosahexaenoic acid (DHA, C22:6, omega-3 fatty acid) representing about 9% of total fatty acid composition. Such considerable concentration making fish oils the richest source of polyunsaturated fatty acids with 5 and 6 double bonds. EPA and DHA are very important omega-3 fatty acids as they play vital roles in building up cell membranes of brain and retina and their deficiency affect negatively their biological functions.

Table 2
Fatty acid composition of flaxseed and fish oils

Fatty Acid	Percentage	
	Flaxseed oil	Fish oil
Myristic (C14:0)	ND****	6.83 ±0.53
Palmitic (C16:0)	5.28 ±0.22	15.25 ±1.1
Palmitoleic (C16:1)	ND	6.57 ±0.57
Stearic (C18:0)	3.33 ±0.12	5.69 ±0.49
Oleic (C18:1)	14.44 ±1.35	27.68 ±1.32
Linoleic (C18:2)	14.27 ±1.66	7.17 ±0.45
Linolenic (C18:3)	62.68 ±3.77	2.95 ±0.09
Eicosenoic acid (C20:1)	ND	2.17 ±0.10
Arachidic acid (C20:0)	ND	3.99 ±0.26
Eicosapentaenoic acid (C20:5)	ND	12.62 ±0.96
Docosahexaenoic acid (C22:6)	ND	9.08 ±0.66
TSF*	8.61 ±0.77	31.76 ±2.05
TUSF**	91.39 ±4.99	68.24 ±3.45
US/S***	10.61 ±1.61	2.15 ±0.02

TSF* = Total Saturated Fatty Acids; TUSF**= Total Unsaturated Fatty Acids; US/S***= Total Unsaturated Fatty Acids / Total Saturated Fatty Acids; ND**** = not detected

Unfortunately, despite the important biological functions that flaxseed and fish oil can perform these functions are limited by the ready susceptibility of such oils (with their high concentrations of unsaturated fatty acids) to oxidation [7]. So, the next step in the protocol was to find the most promising natural antioxidant to be used in stabilizing the flaxseed and fish oils.

3.3. Extraction of rosemary phenolic compounds

3.3.1. Traditional solvent extraction

Rosemary (*Rosmarinus officinalis* L.) leaves is one of the richest sources of phenolic compounds and natural antioxidants with very important health benefits [40-42, 21, 10] To explore the suitability of different extracting solvents with different polarity, we have compared the yield, total phenol content (TPC) and radical scavenging activity (RSA %) of n-hexane, methanol and water extracts at ambient temperature 25 ±1ºC. So, we investigated extraction of the naturally occurring phenolic compounds using three solvents of increasing order of polarity namely: n-hexane (non-polar), methanol (moderate polarity) and distilled water (highly polar). Selection of best solvent was dependent on total yield, total phenolic content, and the radical scavenging activity (RSA %) of its respective extract.

3.3.1.1. Total yield and total phenolic content (TPC)

As presented in Figure 1, methanol gave the highest extract yield (5.5%) followed by water (4.05%) while hexane had the least extract yield (1.2%). Our surprise was that water gave more than threefold total phenolics (13.2 mg GAE/ g dry plant) compared to that of methanol extract (3.5 mg GAE/ g dry plant) as shown in Figure 2. These results may be interpreted as reported by Al-Matani *et al.* [43] who stated that total phenolic content is solvent dependent and is independent on total yield. In agreement with our results Barchan and coworkers [44] reported that phenolic content of three Mentha species extracts increased with the polarity of the solvent; the highest amount of total phenolic content corresponded to water and methanol (polar solvents) extracts whereas n-hexane and dichloromethane (non-polar solvents) extracts showed negligible antioxidant activity.

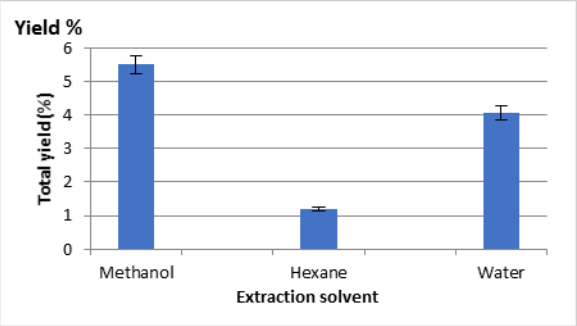

Figure 1, Yield % of rosemary leaves extract using solvents of different polarities.

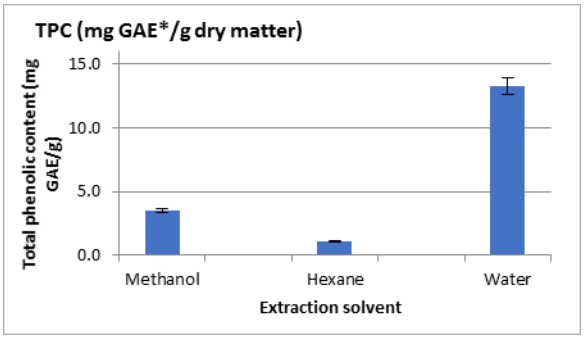

Figure 2, Total phenolic content (TPC) of rosemary leaves extract using solvents of different polarities, (GAE*= Gallic Acid Equivalent).

3.3.1.2. Radical scavenging activity (RSA%) using DPPH assay

Free radicals are known to be a major factor in biological damages. The DPPH radical scavenging assay is a widely used method to evaluate the ability of plant extracts to scavenge free radicals generated from DPPH reagent [24, 45]. DPPH, a stable free radical with a purple color, changes into a stable yellow compound on reacting with an antioxidant. Figure 3 revealed the radical scavenging activity (RSA %) of the different rosemary extracts compared to RSA % of the effective synthetic antioxidant butylated hydroxyl toluene (BHT). As can be seen in Figure 3 the RSA% of the different extracts were significantly different (p<0.05) from each other and went in an increasing order of n-hexane < methanol < BHT < water. Methanol gave acceptable activity (52.79%) compared to that of BHT (63.31%) and water gave the highest activity (84.80%), while n-hexane revealed the least activity (27.14%). It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their

effectiveness to be donors of hydrogen atoms or electrons and to capture the free radicals [46]. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic compounds [47-50].

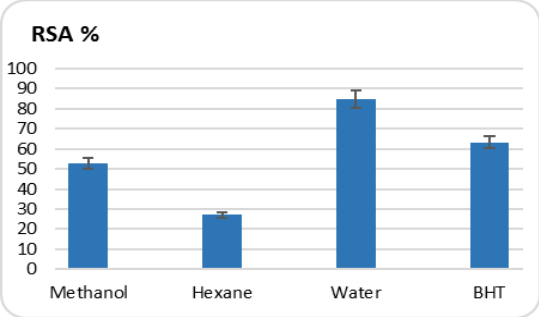

Figure 3, Radical scavenging activity (RSA %) of different rosemary leave extracts by traditional solvent extraction.

The high RSA% of rosemary phenolics extracted by water encouraged us to proceed with water as a green and cheapest solvent for the subsequent stage of this study.

3.3.2. Ultrasonic-assisted extraction

Ultrasonic-assisted extraction was carried out to see whether ultrasonic homogenization would affect the extracted phenolic compounds positively or not. As shown in Figure 4**,** the effect of temperature and extraction time was presented. Results showed that all extracts were of comparable or better RSA% than the synthetic antioxidant BHT. Increasing temperature from 25 to 45ºC enhanced RSA % at each individual time of extraction. At 25ºC there was a significant increase in RSA % with increasing time of extraction from 10 min to 20 min followed by slight increase in RSA % with increasing time from 20 to 30 min, probably because 20 min time period was an adequate time for extracting most of the antioxidant compounds. While at 45ºC, gradual increase in RSA % with increasing time was occurred reaching its maximum (93.61%) after 30 min. So, the optimal conditions chosen to extract the most effective extract from dried rosemary leaves was 1:10 solid to water ratio, and ultrasonic-assisted extraction at 45°C for 30 min.

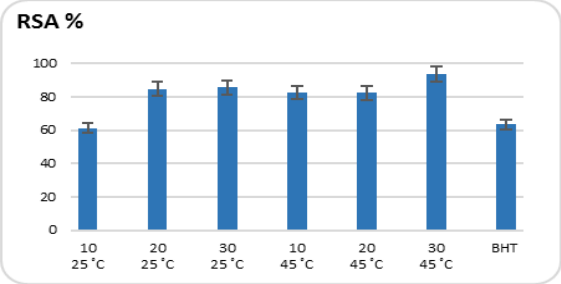

Figure 4, Effect of ultrasonic-assisted water extraction on RSA %.

From the above mentioned results it was seen that the radical scavenging activity of methanol extract, water extract, and BHT gave RSA of about 53%, 85%, and 63%, respectively. So, water or methanol rosemary extracts could represent natural candidates for stabilizing the functional omega-3 oils (flaxseed and fish oils) against oxidation that could substitute for synthetic antioxidant with all its probable hazards [51]. So, the second step of the present work was to examine the antimicrobial activity of the water and methanolic extracts against pathogenic Gram positive and Gram negative bacteria (see material section) and two infectious fungi species (*Aspergillus flavus* and *Aspergillus niger*). The effect of the rosemary extracts was also tested against some beneficial lactobacilli bacteria (see material section).

3.3.3. Antimicrobial activity of rosemary extracts

Although water extract gave the highest amount of total phenolic compounds (Figure 2) as well as highest RSA % (Figure 3), but this water extract had non-detectable activity against the tested pathogens at all concentrations used in the study (0.25 - 15%, data not shown). On the other hand, the methanol extract, which possess lower content of phenolics and lower radical scavenging activity than water extract, revealed observable antimicrobial activity at concentrations from 3 to 15% (Table 3.). In agreement of our results Moreno *et al.* [51] reported that while methanol and acetone extracts of rosemary leaves had broad-spectrum antimicrobial activity and inhibiting the growth of a number of Gram positive and Gram negative bacteria and yeasts by contrast, water extract and pure rosmarinic acid seemed to have a narrow antimicrobial activity. Those authors have returned these differences in antimicrobial activity between water and methanol extract to that

the latter had carnosic acid and carnosol (with high antimicrobial activity) as the main phenolic components of methanol extract while the water extract had rosmarinic acid (with very narrow antimicrobial effect) as the main component. They demonstrated their postulation by the analysis of the phenolic components of both extracts by HPLC and testing the antimicrobial activity by carrying out a qualitative (disk diffusion test) and a quantitative (broth dilution technique) evaluation of the antimicrobial activity of rosemary extracts and pure rosmarinic, carnosic, and carnosol components. As shown in Table 3. Results revealed that the methanol was a suitable solvent for extracting antimicrobial substances from this plant. The highest activity was demonstrated against *Aspergillus flavus, Listeria monocytogenes* and *Yersinia enterocolitica* at the concentration 15% (zone of inhibition 20, 19 and 20 mm respectively) while the lowest activity (zone of inhibition 3 mm) was observed at the concentration 3% against *E. coli* and *Salmonella typhmirum. Pseudomonas aeruginosa* and *Aspergillulls niger* didn't show any inhibition zone at the same concentration. As shown in Table 3, the concentration of 5% of this extract was the lowest concentration that revealed activity against all tested pathogens used. Also, from Table 3 we noticed that the diameter of inhibition zone increased with increasing concentration of plant extract. Our results confirmed also by a number of scientific works [52-54].

Table 3
Antimicrobial effect of rosemary methanol extract.

Pathogenic strains	0.25%	0.50%	0.75%	1.0%	3%	5%	7%	10%	15%
	Diameter of inhibition zone (mm)								
Bacillus cereus	N.D	N.D	N.D	N.D	4^{A}± 0.20	5^{B}± 0.11	7^{C}±0.22	11^{D}±0.14	15^{E}±0.25
Staphylococcus aureus	N.D	N.D	N.D	N.D	4^{A}±0.31	7^{C}±0.31	10^{D}±033	11^{D}±0.19	15^{E}±0.37
Pseudomonas aeruginosa	N.D	N.D	N.D	N.D	N.D	5^{B}±011	6^{B}±0.25	7^{C}±0.27	11^{D}±0.51
Listeria monocytogenes	N.D	N.D	N.D	N.D	8^{C}±0.22	10^{D}±0.37	11^{D}±055	15^{E}±0.33	19^{F}±081
Escherichia coli	N.D	N.D	N.D	N.D	3^{A}±0.38	5^{B}±0.51	7^{C}±0.72	10^{D}±0.65	13^{E}±0.41
Yersinia enterocolitica	N.D	N.D	N.D	N.D	8^{C}±0.91	12^{D}±.70	13^{E}±0.61	13^{E}±0.22	20^{F}±1.10
Salmonella typhimurum	N.D	N.D	N.D	N.D	3^{A}±0.10	5^{B}±0.29	7^{C}±0.38	8^{C}±0.76	11^{D}±0.46
Aspergillusflavus	N.D	N.D	N.D	N.D	7^{C}±0.26	11^{D}±0.63	14^{E}±0.59	17^{EF}±0.48	20^{F}±0.98
Aspergillusniger	N.D	N.D	N.D	N.D	N.D	5^{B}±0.35	6^{C}±0.51	9^{D}±0.54	15^{E}±0.50

Data expressed as (mean ±SD) of 3 replicates;within the same rows, values with different letters are significantly different ($p \leq 0.05$); ND = not detected

3.3.4. The effect of the rosemary extracts on Lactobacilli strains

Table 4. Indicated that the methanol extract had no effect on the survivability of the beneficial bacteria of lactobacilli strains. But the viable count of the most lactobacilli strains decreased approximately 2 log cycles at concentration 7 and 10%. Moreover, the results indicated that *L. plantarum* and *L. helveticus* decreased only one log cycle at concentration 7% compared with other lactobacilli strains. It is clear from the results that lactobacilli strains possessed more resistant to rosemary extract, so, the methanol extract can be used as a preservative agent in different fermented products. Our results agree with Zhang *et al.*, [55].

Table 4
Effect of rosemary methanol extract on lactobacilli.

Lactobacilli strains	Control	3%	5%	7%	10%
	CFU*				
Lactobacillus helveticus	+++	+++	+++	++	+
Lactobacillus acidophilus	+++	+++	+++	+	+
Lactobacillus rhamnosus	+++	+++	+++	+	+
Lactobacillus reuteri	+++	+++	+++	+	+
Lactobacillus casei	+++	+++	+++	+	+
Lactobacillus plantarum	+++	+++	+++	++	+

+++ Bacterial count in $x10^6$; ++ Bacterial count in $x10^5$; + Bacterial count in $x10^4$; CFU* = Colony Forming Units

3.4. Encapsulation efficiency (EE %)

Table 5.Revealed that although EE % for spray dried capsules (90.5 and 91.7%) were higher than that of capsules prepared by extrusion method (87.11 and 88.9%) for fish and flaxseed oils, respectively.

Table 5
Encapsulation efficiency of fish and flaxseed oils encapsulated by extrusion and spray drying techniques.

Samples	Encapsulation Efficiency (%)
Fish oil by spray drying	90.5 ±1.2
Fish oil by extrusion	87.11± 1.25
Flaxseed oil by spray drying	91.7 ± 2.3
Flaxseed oil by extrusion	88.9 ±0.99

Data expressed as (mean ± SD) of 3 replicates.

3.4.1. Antimicrobial effect of microcapsules

From Table 6. Results showed that the fish oil or flaxseed oil had no effect on the survivability of pathogenic strains. The counts of pathogenic strains in negative control ranged between 27 to 61 x 10^5 CFU/ml and the counts were kept on the same log cycle for the positive control for both oil. In the case of microencapsulation of oils, our results indicated that the counts of pathogenic strains decreased around one log cycle for microencapsulation of each oil using extrusion method. This effect of inhibition resulting from rosemary extract not from the oils as we proved before in screening the antimicrobial activity of rosemary methanol extract. In agreement with our results, Mahmoud *et al.* [56] reported that the pores found on the surface of capsules prepared by extrusion method allowing the rosemary extract to exit easily, giving the antimicrobial effect. This result was also confirmed by Gachkar *et al.* [53] and Adam *et al.* [54]. Microencapsulation of oils using spray drying technique had no effect on the pathogenic strains, which were revealed by the comparable count of pathogenic strains to that of the control (~ $x10^5$).

3.5. Production of yogurt using microcapsules

Yogurt samples prepared from fish or flaxseed oils encapsulated by extrusion technique as (Table 7) showed unacceptable characteristics such as appearance, body texture and flavor in the yogurt end products. Therefore, from the practical point of view we recommended not to use the microencapsulation of oil by extrusion technique in the dairy products, especially yogurt. It is worth to mention that the counts of lactic acid bacteria for all samples were comparable to that of the control. On the other hand, yogurt fortified with spray dried oils revealed acceptable characteristics of sensory evaluation as well as the viable count of the beneficial lactic acid bacteria as shown in Table 8. The results of yogurt samples that fortified with each oil encapsulated by spray drying technique found in (Table 8). At zero-time of storage, the counts of lactic acid bacteria in the samples fortified with different capsules was ranged between 6.64 and 7.20 log cfu/mL. These counts although being significantly different ($p<0.05$) from that of control samples (7.9 log cfu/mL) were still close to it. The yogurt samples fortified with fish oil tends to have pale yellow color compared to control, but this color was acceptable in the evaluation. Also, the flavor of capsules was acceptable during sensory evaluation. So, microencapsulation of oil by spray drying technique had positive effects on the sensory evaluation of

yogurt. Our results were confirmed by Tamjidi *et al.* [57] who found that sensory results displayed by trained panelists evaluated overall acceptance of enriched yogurt with treated-fish oil microcapsules.

Table 6
Antimicrobial effect of different microcapsules (CFU/mL).

Pathogenic strains	Control (negative)	Free fish oil (positive)	Free flaxseed oil (positive)	Capsules with fish oil by spray drying method	Capsules with flaxseed oil by spray drying method	Capsules with fish oil by extrusion method	Capsules with flaxseed oil by extrusion method
Bacillus cereus	$30x10^{5A}$ ±0.50	$28x10^{5A}$ ±0.58	$33x10^{5A}$ ±0.80	$24x10^{5A}$ ±0.57	$27x10^{5A}$ ±0.82	$13x10^{4B}$ ±0.55	$16x10^{4B}$ ±0.55
Staphylococcus aureus	$61x10^{5A}$ ±0.85	$58x10^{5A}$ ±0.65	$64x10^{5A}$ ±0.80	$50x10^{5A}$ ±0.60	$54x10^{5A}$ ±0.60	$18x10^{4B}$ ±0.55	$22x10^{4B}$ ±0.58
Pseudomonas aeruginosa	$28x10^{5A}$ ±0.58	$30x10^{5A}$ ±0.60	$31x10^{5A}$ ±0.58	$22x10^{5A}$ ±0.60	$25x10^{5A}$ ±0.65	$12x10^{4B}$ ±0.50	$17x10^{4B}$ ±0.58
Listeria monocytogenes	$45x10^{5A}$ ±0.60	$43x10^{5A}$ ±0.70	$40x10^{5A}$ ±0.65	$38x10^{5A}$ ±0.60	$36x10^{5A}$ ±0.68	$15x10^{4B}$ ±0.58	$20x10^{4B}$ ±0.64
Escherichia coli	$39x10^{5A}$ ±0.72	$41x10^{5A}$ ±0.68	$43x10^{5A}$ ±0.60	$37x10^{5A}$ ±0.60	$38x10^{5A}$ ±0.55	$18x10^{4B}$ ±0.50	$21x10^{4B}$ ±0.56
Aspergillusniger	$27x10^{5A}$ ±0.62	$29x10^{5A}$ ±0.55	$30x10^{5A}$ ±0.58	$25x10^{5A}$ ±0.60	$27x10^{5A}$ ±0.65	$14x10^{4B}$ ±0.58	$11x10^{4B}$ ±0.50

Data expressed as (mean ± SD) of 3 replicates;within the same rows, values with different letters are significantly different ($p \leq 0.05$).

Table 7
Evaluation of final fresh yogurt samples fortified with extrusion microcapsules.

Treatments	pH	Lactobacilli counts (log cfu/mL)	Streptococci counts (log cfu/mL)	Flavor (50)	Body & texture (40)	Appearances (10)
Control yogurt	4.62^{A} ±0.12	7.90^{A} ±0.55	7.10^{A} ±0.68	48^{A} ±0.80	38^{A} ±0.74	9^{A} ±0.72
Yogurt with fish oil capsules	4.73^{B} ±0.10	6.88^{B} ±0.50	6.64^{B} ±0.55	Unacceptable	10^{B} ±0.30	Unacceptable
Yogurt with flaxseed oil capsules	4.71^{B} ±0.11	6.94^{B} ±0.58	6.70^{B} ±0.59	Unacceptable	10^{B} ±0.28	Unacceptable

Data expressed as (mean ±SD) of 3 replicates; within the same column, values with different letters are significantly different ($p \leq 0.05$).

Table 8
Evaluation of final fresh yogurt samples fortified with spry dried microcapsules.

Treatments	pH	Lactobacilli counts (log cfu/mL)	Streptococci counts (log cfu/mL)	Flavor (50)	Body & texture (40)	Appearances (10)
Control yogurt	4.62^{A} ±0.11	7.90^{A} ±0.45	7.10^{A} ±0.55	48^{A} ±0.68	38^{A} ±0.48	9^{A} ±0.38
Yogurt with fish oil capsules	4.55^{B} ±0.10	7.20^{B} ±0.42	6.64^{C} ±0.49	47^{A} ±0.58	37^{AB} ±0.40	9^{A} ±0.35
Yogurt with flaxseed oil capsules	4.60^{A} ±0.10	7.00^{B} ±0.40	6.70^{B} ±0.52	47^{A} ±0.46	38^{A} ±0.45	9^{A} ±0.38

Data expressed as (mean ± SD) of 3 replicates; within the same column, means with different letters are significantly different ($p \leq 0.05$).

Moreover, during storage of yogurt (Tables 9 and 10), the counts of lactic acid remain in the same log for all treatments. Also, the counts of bacterial strains not negatively affected by the microencapsulation of oils during storage period for 10 days. The counts of starter bacteria ranged between 7.00 and 8.05 log cfu/mL at 10 days of storage. Table 10, showed that the sensory evaluation during storage still accepted where the flavor after 10 days of storage had scores of 43 and 44 for yogurt samples fortified with fish and flaxseed oil, respectively and the flavor of control reached to 46. In addition, results showed reduction in the body and texture score during storage, resulted from appearance of small amount of water content in all samples at the end of storage. This syneresis may be resulted from reduction of pH values during storage. From our results, it can be concluded that the production of fish oil and flaxseed oil microcapsules by spray drying technique is a promising procedure for functional food candidates such as yogurt. Our results agree with Ghorbanzade *et al.*, [58] who reported that adding nano-encapsulated fish oil into yogurt gave closer characteristics to control sample in terms of sensory characteristics than yogurt fortified with free fish oil.

Table 9

Evaluation of final yogurt samples fortified with spray dried microcapsules at 5 days of storage.

Treatments	pH	Lactobacilli counts (log cfu/mL)	Streptococci counts (log cfu/mL)	Flavor (50)	Body & texture (40)	Appearance s (10)
Control yogurt	$4.56^{A} \pm 0.12$	$8.05^{A} \pm 0.39$	$7.95^{A} \pm 0.59$	$48^{A} \pm 0.44$	$36^{A} \pm 0.46$	$9^{A} \pm 0.33$
Yogurt with fish oil capsules	$4.51^{A} \pm 0.10$	$7.90^{B} \pm 0.37$	$6.93^{B} \pm 0.49$	$46^{B} \pm 0.45$	$35^{A} \pm 0.41$	$9^{A} \pm 0.30$
Yogurt with flaxseed oil capsules	$4.56^{A} \pm 0.09$	$7.60^{B} \pm 0.40$	$7.00^{B} \pm 0.52$	$47^{A} \pm 0.42$	$35^{A} \pm 0.43$	$9^{A} \pm 0.30$

Data expressed as (mean ± SD) of 3 replicates; within the same column, means with different letters are significantly different ($p \leq 0.05$).

Table 10

Evaluation of final yogurt samples fortified with spry drier microcapsules at 10 days of storage.

Treatments	pH	Lactobacilli counts (log cfu/mL)	Streptococci counts (log cfu/mL)	Flavor (50)	Body & texture (40)	Appearances (10)
Control yogurt	$4.51^{B} \pm 0.17$	$8.30^{A} \pm 0.39$	$7.10^{AB} \pm 0.45$	$46^{A} \pm 0.40$	$35^{A} \pm 0.43$	$7^{A} \pm 0.32$
Yogurt with fish oil capsules	$4.55^{B} \pm 0.18$	$8.00^{AB} \pm 0.42$	$6.89^{B} \pm 0.43$	$43^{C} \pm 0.36$	$33^{B} \pm 0.40$	$7^{A} \pm 0.30$
Yogurt with flaxseed oil capsules	$4.60^{A} \pm 0.21$	$7.80^{B} \pm 0.40$	$7.23^{A} \pm 0.43$	$44^{B} \pm 0.32$	$34^{A} \pm 0.45$	$7^{A} \pm 0.30$

Data expressed as (mean ± SD) of 3 replicates; within the same column, means with different letters are significantly different ($p \leq 0.05$).

3.6. Oxidative Stability of Microcapsules

The oxidative stability of the oils entrained in microcapsules prepared by extrusion and spray drying techniques were evaluated by measuring the primary oxidation products represented by conjugated diene value and conjugated triene value as well as measuring the secondary oxidation products represented by the *p*-anisidine value. Tables (11, 12) revealed that although storage at refrigerator temperatures showed good oxidative stability as measured by conjugated diene value for both encapsulated fish or flaxseed oils prepared by the extrusion method, yet, its shelf life deteriorate rapidly at room temperature. Whereas, spray drying method had pronounced effect on enhancing its oxidation stability throughout the whole period of storage (37 days) either in refrigerator or at room temperature. Moreover, samples were kept with acceptable records after more than 5 months (data not shown).Tables (13, 14) presented the *p*-anisidine values (which measure the amount of aldehydes and ketones produced as a result of primary oxidation products dissociation) of the entrained oils in microcapsules prepared either by extrusion or spray drying methods. Results indicated that samples prepared by extrusion technique have been deteriorated very rapidly either for fish or flaxseed oils both in refrigerator and at

room temperatures and exceeded the recommended values by the international safety standards at anisidinevalue < 20 [59]. Again, spray dried microcapsules entrained oils that survive throughout the whole period of storage without oxidation rancidity as shown by its acceptable anisidine value either in refrigerator or at room temperature, Tables (13, 14).

Table 11

Conjugated dienes and trienes of fish oil encapsulated by extrusion and spray drying techniques stored at room temperature and in refrigerator.

Storage period	Extrusion				Spray drying			
	Conjugated Dienes		Conjugated Trienes		Conjugated Dienes		Conjugated Trienes	
	R .T.	Ref.	R .T.	Ref.	R .T.	Ref.	R .T.	Ref.
Zero Time	1.67 ±0.12	1.67 ±0.12	0.33 ±0.01	0.33 ±0.01	0.23 ±0.005	0.23 ±0.005	0.10 ±0.003	0.10 ±0.003
After 10 days	3.77 ±0.28	1.72 ±0.18	0.54 ±0.02	0.35 ±0.04	0.27 ±0.012	0.23 ±0.008	0.11 ±0.010	0.10 ±0.008
After 21 days	4.99 ±0.57	1.74 ±0.00	0.99 ±0.14	0.45 ±0.00	0.35 ±0.015	0.28 ±0.014	0.15 ±0.015	0.11 ±0.014
After 29 days	5.76 ±0.28	1.28 ±0.07	0.77 ±0.03	0.32 ±0.01	0.48 ±0.049	0.32 ±0.006	0.15 ±0.005	0.11 ±0.012
After 37 days	5.99 ±0.42	1.32 ±0.2	1.05 ±0.01	0.58 ±0.06	0.67 ±0.053	0.33 ±0.012	0.17 ±0.013	0.12 ±0.015

Each value represents mean ±SD (n = 3), R.T. = Room Temperature, Ref. = Refrigerator.

Table 12

Conjugated dienes and trienes of flaxseed oil encapsulated by extrusion and spray drying techniques stored at room temperature and in refrigerator.

Storage period	Extrusion				Spray drying			
	Conjugated Dienes		Conjugated Trienes		Conjugated Dienes		Conjugated Trienes	
	R .T.	Ref.	R .T.	Ref.	R .T.	Ref.	R .T.	Ref.
Zero Time	0.62 ±0.18	0.62 ±0.18	0.023 ±0.001	0.023 ±0.001	0.15 ±0.03	0.15 ±0.03	0.02 ±0.001	0.02 ±0.001
After 10 days	1.86 ±0.21	1.46 ±0.23	0.34 ±0.07	0.15 ±0.04	0.17 ±0.04	0.15 ±0.03	0.02 ±0.002	0.02 ±0.001
After 21 days	2.82 ±0.09	1.54 ±0.24	0.69 ±0.03	0.20 ±0.04	0.22 ±0.01	0.15 ±0.02	0.03 ±0.004	0.02 ±0.001
After 29 days	2.49 ±0.10	0.94 ±0.02	0.60 ±0.01	0.22 ±0.06	0.22 ±0.02	0.16 ±0.03	0.04 ±0.002	0.02 ±0.002
After 37 days	3.56 ±0.18	1.31 ±0.26	0.71 ±0.04	0.27 ±0.03	0.26 ±0.04	0.16 ±0.03	0.05 ±0.003	0.03 ±0.002

Each value represents mean ± SD (n = 3), R. T. = Room Temperature, Ref. = Refrigerator.

Table 13

Anisidine value of fish oil encapsulated by extrusion and spray drying techniques stored at room temperature and in refrigerator.

Storage period	Extrusion		Spray drying	
	R .T.	Ref.	R .T.	Ref.
Zero Time	45.6 ±1.15	45.6 ±1.15	13.22 ±0.49	13.22 ±0.49
After 10 days	327.2 ±2.54	118.8 ±1.61	13.26 ±0.45	13.20 ±0.20
After 21 days	327.2 ±3.72	145.2 ±2.03	13.30 ±0.44	13.23 ±0.29
After 29 days	466.23 ±2.84	165.11 ±2.01	14.30 ±0.44	13.41 ±0.35
After 37 days	512.4 ±2.51	180.9 ±2.10	15.33 ±0.41	13.44 ±0.25

Each value represents mean ± SD (n = 3), R. T. = Room Temperature, Ref. = Refrigerator.

Table 14

Anisidine value of flaxseed oil encapsulated by extrusion and spray drying techniques stored at room temperature and in refrigerator.

Storage period	Extrusion		Spray drying	
	R .T.	Ref.	R .T.	R .T.
Zero Time	19.92 ±1.20	19.92 ±1.20	8.56 ±0.56	8.56 ±0.56
After 10 days	81.7 ±1.50	32.3 ±1.80	9.35 ±1.05	8.60 ±0.44
After 21 days	165.43 ±2.70	40.3 ±1.50	9.90 ±2.00	9.11 ±1.30
After 29 days	248.47 ±2.35	45.9 ±1.55	10.70 ±0.95	9.20 ±0.56
After 37 days	255.2 ±3.00	60.4 ±1.66	11.00 ±0.65	9.50 ±0.45

Each value represents mean ± SD (n = 3), R. T. = Room Temperature, Ref. = Refrigerator.

4. Discussion

Our main objective is to prepare stabilized multi-functional microencapsulated omega-3 oils enriched with efficient natural antioxidants (from rosemary extract). These microcapsules can be fortified into functional foods (dairy products, beverages, or baby formulations and pharmaceuticals to increase the bioavailability and functionality of omega-3 oils.

To achieve this goal, we chose two omega-3 rich oils (flaxseed and fish oils). Because one of the most challenges facing omega-3 rich oils is its very susceptibility to be oxidized we thought to overcome this main challenge by two approaches, namely encapsulation and fortification with strong natural antioxidants. Our accumulated experience in the field of natural antioxidants encouraged us to use one of the strongest natural antioxidants bearing plants, the rosemary (*Rosmarinus officinalis* L.), to be the candidate in retarding oxidation of these sensitive oils together with protecting such sensitive oils also through encapsulation with edible polymers either by extrusion method or using spray drying technique.

We expected first that these two different methodologies would work well because both of them could construct a line of defence against invading oxygen. But the experiment learned us that spray drying went more favorable toward protecting the entrained oils against oxidation probably due to different wall materials used in both techniques (maltodextrine and Arabic gum in case of spray drying or sod alginate and Arabic gum in extrusion method) such different wall materials could behave differently in protecting its core. Also, results revealed that spray dried microcapsule could be incorporated successfully in sensitive dairy products such as yogurt as it could obscure the unpleasant odor of the very important oil like fish oil and at the same time behaved like normal control yogurt. So, this work made a base of preparing fortified or functional foods that could be one of the important commodities for peoples seeking good health.

To explore the suitability of natural rosemary phenolic extracts to stabilize flaxseed and fish oils against oxidation different solvents with different polarities have been investigated. Results demonstrated that although methanol gave the maximum recovery percentage (yield %), water extract showed the highest total phenolic content followed by methanol extracts while n-hexane revealed the least yield, total phenolic and RSA %.

When the different extracts were compared with the antioxidative potentials of the reference synthetic antioxidant, BHT, our results showed that the decreasing trend of RSA % was water extract > BHT > methanol extract > hexane extract. However, when ultrasonic-assisted extraction technique was used with distilled water as a solvent at different temperatures (25 and 45°C) and different time intervals (10, 20, and 30 min) the potency of the extract as radical scavenger were greatly enhanced specifically at 45°C and 30 min).

The antimicrobial activity of both water and methanol extracts of rosemary leaves were tested against some pathogenic bacteria (Gram negative and Gram positive), some fungi, as well as against some

beneficial probiotic bacteria (some Lactobacilli species) to select the extract that will be the proper one to proceed with for preparing the microcapsules. Results indicated that although water extract gave highest total phenolics and highest radical scavenging activity, but, it had no antimicrobial activity against both Gram negative or Gram positive bacteria while methanol extract revealed inhibitory activity against all tested pathogens at concentration of 5 % or higher. Results indicated that the methanol extract had no effect on the survivability of the beneficial bacteria of lactobacilli strains. But the viable count of the most lactobacilli strains decreased approximately 2 log cycles at concentration 7 and 10%. Results showed that lactobacilli strains possess more resistant to rosemary extract which make the methanol extract capable to be used as preservative agent in different fermented products.

5. Conclusion

Omega-3 rich oils are extremely important from the health point of view either for adults, children or foetuses. Therefore, we tried in the present work to maximize its functionality and bioavailability through fortification with effective natural antioxidants and encapsulation with promising wall materials. So, different extracts of rosemary leaves were prepared of which methanol extract has been proved to be of superior activity either as oxidative radical scavenger or as antimicrobial entity. Maltodextrin and Arabic gum were shown to be active wall materials for the prepared microcapsules. Also, our research let the door opened for testing other biodegradable natural wall materials to be utilized in preparing microcapsules ready for food and nutraceutical stuff. This study spotlighting the effectiveness of spray drying technique to formulate microcapsules capable to be incorporated in delicate foods like yogurt.

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7. Conflicts of interest:

There is no any Conflict of Interest

8. References

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