

Preparation and Characteristics of Micro and Nano Wheat Germ Oil Capsules

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Abstract: Encapsulation technology offers the potential to significantly improve the solubility and bioavailability of many functional ingredients. Wheat germ oil (WGO) was extracted from the germ of the wheat kernel. WGO contains omega-6 (44–65 %), omega-3 (4–11 %) fatty acids. Due to its highly content of polyunsaturated fatty acids, WGO is highly prone to oxidation of acids. The main goal of this work was to increase the efficiency and stability of Wheat germ oil using encapsulation techniques (Micro and Nanocapsulation) and study the effect of these techniques on the Transmission electron microscopy, viscosity, particle size and zeta potential, acidity, TBARs and peroxide value on micro and nano WGO emulsions. Also, the quality of micro and nano WGO capsules for application in food industry was studied. The results indicated that, WGO has been reported to be a good source of polyunsaturated fatty acids such as oleic and linoleic acids which have health benefits. Nano WGO (ultrasonication) had the lowest value of PV (59.78 μ g Fe/ml) after ten days of storage period. TBARs value was increased from 0.87 to 0.91 mg MDA/ L in nano WGO emulsion with low differences; 0.89 to 1.01 mg MDA/L in micro WGO emulsion at the end of storage at 55°C. Nano technique was reported to have a slightly lower acidity than micro technique. Micro technique highly ESI increased among these values during storage periods compared to zero time. It can be said that nano technique (ultrasonication) has a positive impact on emulsification. While the majority of the particles was 71.41 μ m for nano WGO emulsion (ultrasonicated sample), for the sample which was homogenized (micro WGO emulsion), was 694.3 μ m. From the Transmission electron microscopy image of micro and nano WGO emulsions, the size of nano WGO emulsion was 10 nm. As the viscosity of the micro and nano WGO emulsions decreased, the encapsulation efficiency values also decreased. This result showed that, ultrasonic homogenization gives better efficiency and quality results in capsulation of WGO.

Key words: Wheat germ oil • Spray Drying • Ultrasonication Process • In Vitro Digestion • Quality Characteristics • Transmission Electron Microscopy

INTRODUCTION

Vegetable oils are important component of humane diet. Wheat germ is one of the main by-products of the wheat milling industry. Wheat germ has high nutritional value, containing seven times more fat, three times more protein, fifteen times more sugar and six times more dietary fibre than standard white flour [1]. Wheat germ oil (WGO) has a high nutritional and health benefits like reducing plasma and liver cholesterol levels [2]. It is known to have omega-6 (44–65 %), omega-3 (4–11 %) fatty acids and about 80% of the total fatty acids in WGO are unsaturated. linoleic acid (18:2) represented 57-58% of the total fatty acids [3, 4]. Due to its highly content of

polyunsaturated fatty acids, wheat germ oil is highly prone to oxidation of acids [5]. Micro and Nano encapsulation has a great potential to solving this problem. They protect these molecules from environmental factors such as pH, oxygen, light etc., serving as barrier between the molecule and the environment [6]. They were also used in the oil industry for products in additives formulations for different applications [7]. Nanocapsules are solid, hollow particles ranging from 10 - 1,000 nm in diameter. Recently, they have been used in drug-delivery systems [8, 9]. Proteins and carbohydrates are frequently studied as matrices to encapsulate lipophilic compounds by spray drying, which is a one-step, low-energy and economical encapsulation

method commonly used in the food industry [10]. Mixtures of whey protein (WP) and maltodextrin (MD) have been used to encapsulate, Whey protein powder is important compounds used in encapsulation techniques, it is known to aggregate during heating, especially at increased ionic strength and acidity near isoelectric point when electrostatic repulsion weakens [11]. Arabic gum (gum acacia) has been the standard of excellence as a flavour encapsulating material for many years. It is an excellent emulsifier, bland in flavour and provides good retention properties for the volatiles during the drying process [12].

This study aimed to increase the efficiency and stability of Wheat germ oil using encapsulation techniques (Micro and Nano-capsulation) and study the effect of these techniques on the transmission electron microscopy, viscosity, particle size and zeta potential, acidity, TBARs and peroxide value on micro and nano WGO emulsions. Also, the quality of micro and nano WGO capsules for application in food industry was studied.

MATERIALS AND METHODS

Materials: Wheat germ was obtained from private wheat mills Company, Tanta City, Gharbiya Governorate, Egypt. Arabic gum (gum acacia) was obtained from Lobal Chemei Company. Maltodextrin (DE16) used in this study was obtained from Sigma- Alderch Company. Whey protein concentrate containing 81% protein was obtained from Lobal Chemei Company.

Methods

Preparation of Wheat Germ: Wheat germ (WG) was ground in a laboratory mill (Moulinex, model No 205, France). Ground wheat germ collected in polyethylene bags until further use for extraction test.

Extraction of Wheat Germ Oil (WGO): The ground wheat germ was extracted according to the method of AOAC [13] at Food Technology Department, National Research Centre, Dokki, Giza Governorate, Egypt, by petroleum ether (40-60°C, Pb) as a solvent using a Soxhlet apparatus. The solvent was separated with a rotary vacuum evaporator (R- 300, Fisher scientific) at 50°C.

Micro and Nano WGO Emulsions Preparation: Micro and Nano WGO emulsions were prepared according to Wang *et al.* [14] at Food Technology Department,

National Research Centre, Dokki, Giza Governorate, Egypt. Maltodextrin (10%) and gum Arabic (10%) were dissolved in distilled water by gentle magnetic stirring at 50 ~ 60°C for 1 h. WP was dissolved in 0.1M sodium phosphate buffer pH 7.0 by gentle magnetic stirring at 60 ~ 80°C for 30 min until completely dissolved. Maltodextrin and WP were mixed in ratios 4:1 by gentle magnetic stirring for 1 h. A half gram of Arabic gum was added into above solution and homogenized at Ultra-Turrax homogenizer T18 basic (IKA, Wilmington, USA), operating at a speed of 12,000 rpm for 5 minutes. Then, WGO was added to the wall materials (maltodextrin, WP and Arabic gum) in ratio 1:4. The emulsification process was performed in two stages of homogenization. First, pre micro emulsion was homogenized using an Ultra-Turrax homogenizer (IKA, Wilmington, USA), operating at a speed of 16,000 rpm/5min. Second, pre nano emulsion was homogenized by using Ultrasonic Homogenizer (UP650, Acculab, USA) at a speed of 22,000 rpm/5min. The ultrasonication process was performed at 28 ~ 30°C for 40minutes at 31% power.

Spray Drying: Micro and Nano emulsions of wheat germ oil were spray dried (Mini spray dryer B-290, BÜCHI Labortechnik, Switzerland) by a nozzle atomization system with 1.5 mm diameter nozzle and main spray chamber of 500×215 mm. The solution of the capsules was fed into the main chamber through a peristaltic pump and the feed flow rate was controlled by the pump rotation speed. Drying air flow rate was 85% and compressor air pressure was 0.06 MPa. Inlet and outlet air temperature were 150°C and 80°C, respectively and feed flow rate was 70% according to Charve and Reineccius [10]. The powdered Micro and Nano capsules were collected and stored in desiccator containing calcium chloride at 25°C to prevent moisture absorption and lump formation until further studies.

Analysis Techniques

Fatty Acids composition of Wheat Germ Oil: Fatty acid methyl esters (FAME) were analyzed by gas chromatography (Agilent 7820A, USA) using a flame ionization detector (FID) system to identify and quantify the each individual fatty acids of wheat germ oil. Fatty acids composition of the oil was determined according to the method described in AOCS [15]. The retention times of each FAME were compared against the standard mixture of FAME to identify the FAME composition of the sample. Fatty acids composition results were expressed as weight percentage.

Characteristics of Micro and Nano Emulsions

Peroxide Value (PV): Emulsions were held in disposable centrifuge polypropylene tubes (Fisher Scientific, Pittsburg, PA, USA) and incubated in the dark at 55°C for 10 days, with measurements being taken every 2 days. Lipid hydro peroxides were measured using a method adapted from Pegg [16]. The absorbance was measured at 510 nm using an UV-visible spectrophotometer (BioTek, Synergy HT, USA). Concentrations of PV were calculated as µg using a calibration curve of (Fe³⁺) iron III chloride.

Thiobarbituric Acid-reactive Substances (TBARS):

Emulsions were held in disposable centrifuge polypropylene tubes (Fisher Scientific, Pittsburg, PA, USA) and incubated in the dark at 55°C and measured every 5 days for 20 days using the method of Lynch and Frei [17]. The absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were calculated as mg MDA/L (TBA units), using a standard curve of 1,1,3,3-tetraethoxypropane.

pH value: The pH values of emulsions were determined according to the method described by Alakali *et al* [18]. Emulsions were shaken with 100 ml water, filtered and the pH of filtrate was measured every 3 days for 15 days at room temperature using a digital pH- meter (JENWAY, Model No. 3510, UK).

Acidity: Free Fatty acids (FFA) value as an index of fat hydrolyses was determined every 3 days for 15 days as described by AOCS [15]. The percentage of free fatty acids was calculated as oleic acid (%) from the following equation:

$$\text{Free fatty acid (FFA) (\%)} = \frac{V \times N \times 28.2}{W}$$

where:

V = volume of NaOH

N = normality of NaOH

W = weight of fat in 25 ml chloroform extract.

Acid value was calculated by using the equation

Acid value = FFA x 1.99

Emulsion Stability Index (ESI): The stability of the emulsion was determined by measuring the water volume fraction, Hermanto *et al* [19]. 100 mL of each emulsion were transferred gradually to 100 ml cylinders, then capped and stored for 5, 10, 15, 20 and 25 days, at 25°C. The emulsions were monitored by visual observation of

the height of the water layer formed at the upper of the cylinders. The emulsion stability index (ESI) was calculated using the following equation:

$$\text{Emulsion stability index (ESI) (\%)} = \frac{1 - \text{Total volume of the separated water layer}}{\text{Total volume of the oil layer in the inclusion complex}}$$

Particle Size and Zeta Potential: The size distribution of the emulsions droplet was determined according to the method of Malaki Nik *et al* and Jessie [20, 21], using a light scattering instrument (Surface Zeta Potential and Particle Size Analyzer (ELS-8000, Otsuka Electronics, Japan).

Viscosity: The measurement of viscosity was performed determined according to the method described by ASTM [22], by viscometer (Brookfield, DV1, USA), with temperature controlled at 25°C. Dynamic viscosity was reported in centipoise.

Transmission Electron Microscopy (TEM): Morphology samples of emulsion was performed according to the method described by Cruz *et al* [23], examined by transmission electron microscopy (JEM-1400, 160 kV, USA).

Characteristics of Wheat Germ Oil Micro and Nano Capsules

Encapsulation Efficiency (EE): Encapsulation efficiency (EE) was determined according to the method described by Bae and Lee [24]. Fifteen milliliters of hexane were added to 1.5 g of powder in a glass jar with a lid, which was shaken by hand for the extraction of free oil, during 2 min, at room temperature. The solvent mixture was filtered through a whatman filter paper no.1 and the powder collected on the filter paper was rinsed three times with 20mL of hexane. Then, the solvent was left to evaporate, 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. Encapsulation efficiency (EE) was calculated from equation.

$$EE = \left(\frac{T0 - S0}{T0} \right) \times 100$$

where

T0 is the total oil content.

S0 is the surface oil content.

In vitro Digestion: The digestion of the samples, under simulated gastrointestinal tract conditions, was conducted with slight modifications of a previously described method, Jessie [21]. About, 1.5 g of powder was mixed with 13.5 mL of basal saline (140 mM NaCl, 5 mM KCl and 150 M BHT) for 10 min. To initiate the gastric digestion, the mixture was treated with 4.5 mL simulated gastric fluids (SGF) (containing 3.2 g/L pepsin in 1 M HCl), followed by adjusting pH to 2.0 using 1.0 M NaOH. After 1 h incubation at 37°C, the pH of the sample was adjusted to 7.5 with 1.0 M NaOH. Then, 4.5 mL simulated intestinal fluid (SIF) (containing 4.76 mg/mL pancreatin and 5.16 mg/mL porcine bile extract, pH 7.5) was added. During 2 h of the intestinal digestion process, to neutralize the free fatty acids (FFAs) released from lipid digestion, the pH of the solution was maintained at 7.5 by adding 0.1 M NaOH manually. The volume of NaOH added over time was recorded throughout the digestion. The percentage of FFAs released (%) during the digestion process time (every 30 min) was calculated.

Statistical Analysis: Data are presented in Tables as means \pm standard deviation (SD). Values were statistically analyzed by one-way analysis of variance (ANOVA) according to Sendecor and Cochran [25] by using SPSS version 11.0, Chicago, USA software package.

RESULTS AND DISCUSSION

Chemical Analysis

Fatty Acids Composition of Wheat Germ Oil: Fatty acids are important components of lipids which can have an important influence on human plasma cholesterol level. For instance, saturated fatty acids increase cholesterol levels in contrast to total monounsaturated fatty acids (MUFA) and total polyunsaturated fatty acids (PUFA) [26]. Data in Table (1) show the fraction of WGO which reveal that the highest saturated fatty acids was palmitic acid giving the value of 13.10 (%). From the obtained results of the same Table, it could be noticed that the content of total saturated fatty acids was (16.05 %). While, the highest reading was scored by total unsaturated fatty acids (78.94 %). WGO has been reported to be a good source of polyunsaturated fatty acids such as oleic and linoleic acids which have health benefits. Fatty acids composition of wheat germ oil were 20.14% of saturated fatty acids and 79.86% of unsaturated fatty acids, where the saturated: unsaturated ratio were 1:3.96 respectively [27]. As the amount of PUFA content increases, an oxidation reaction of oil is more likely to

Table 1: Fatty acids composition (%) of Wheat Germ Oil

Common Name	Scientific Name	%
Palmitic acid	Hexadecanoic acid C _{16:0}	13.10
Stearic acid	Octadecanoic acid C _{18:0}	2.50
Arachidic acid	Eicosanoic acid C _{20:0}	0.30
Behenic acid	Docosanoic acid C _{22:0}	0.15
Total Saturated fatty acid		16.05
Palmitoleic acid	9-Hexadecenoic acid C _{16:1}	0.21
Oleic acid	9-Octadecenoic acid C _{18:1}	24.60
Linoleic acid	9,12-Octadecadienoic acid C _{18:2}	51.90
Linolenic acid	9,12,15-Octadecatrienoic acid C _{18:3}	2.07
Gadoleic acid	9-Eicosenoic acid C _{20:1}	0.16
Total unsaturated Fatty acids		78.94
Unknown		5.01
PUFA/ SFA ratio		3.36

USFA/ SFA unsaturated fatty acids to saturated fatty acids ratio

occur. In this respect, Mehmet *et al.* [28] reported that the percentages of palmitic, oleic, linoleic and linolenic acids determined in the cold pressed wheat germ oil were 15.89, 15.48, 54.88 and 7.34% of total fatty acids, respectively. The relationship between saturated and polyunsaturated FA content is expressed as P/S index. This value is an important parameter for determination of nutritional value of certain oil. Oils and fats with higher value of P/S index than 1 are considered to have nutritional value [29]. Polyunsaturated fatty acids to saturated fatty acids P/S index of WGO under study was 3.36, which make oil the most suitable edible oils for mass consumption.

Characteristics of Micro and Nano Emulsions

Peroxide Value (PV): Peroxide value is a standard index to monitor lipid deterioration. The PV is a measure of all peroxides and other lipid oxidation products that form during primary oil oxidation. High PV indicates low oil quality [30]. Fresh oils contain insignificant peroxides due to the self-protection via the presence of certain natural antioxidant [31]. From the data in Table (2), it could be observed that, peroxide value of micro and nano WGO emulsions at zero time was 1.49 and 0.83 μ gFe/ml, respectively. This number of PV was low; suggest that oil emulsions can be stored for a long period without deterioration. From the same table, PV was highest in micro WGO emulsion (69.25 μ gFe/ml), while PV in nano WGO emulsion (ultrasonication) was (59.78 μ gFe/ml) at the end of storage (10 days) at 55°C. The high value of PV is indicative of high levels of oxidative rancidity of the oil.

Thiobarbituric Acid-Reactive Substances (TBARS):

TBA-test is a sensitive test for the decomposition products of highly unsaturated fatty acids which do not appear in peroxide determination [32]. From Table (3),

Table 2: Peroxide value ($\mu\text{gFe/ml}$) of micro and nano wheat germ oil emulsions

Treatment	Storage period (day)					
	0	2	4	6	8	10
M	1.49 \pm 0.028	21.15 \pm 0.808	104.55 \pm 1.813	75.42 \pm 0.759	77.62 \pm 0.689	69.25 \pm 0.778
N	0.83 \pm 0.008	15.94 \pm 0.521	103.42 \pm 1.756	78.23 \pm 0.638	77.42 \pm 0.732	59.78 \pm 0.543

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

Table 3: Thiobarbituric acid-reactive substances (mg MDA/ L) of micro and nano wheat germ oil emulsions

Treatment	Storage period (day)				
	0	5	10	15	20
M	0.87 \pm 0.004	0.89 \pm 0.030	0.93 \pm 0.022	0.99 \pm 0.008	1.01 \pm 0.014
N	0.84 \pm 0.016	0.87 \pm 0.016	0.88 \pm 0.005	0.89 \pm 0.042	0.91 \pm 0.063

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

Table 4: pH values of micro and nano wheat germ oil emulsions

Treatment	Storage period (day)					
	0	3	6	9	12	15
M	6.37 \pm 0.028	6.37 \pm 0.097	6.33 \pm 0.075	6.29 \pm 0.097	6.28 \pm 0.047	6.27 \pm 0.085
N	6.46 \pm 0.096	6.43 \pm 0.085	6.41 \pm 0.012	6.40 \pm 0.081	6.38 \pm 0.091	6.38 \pm 0.063

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

Table 5. Acid value (mg /g) of micro and nano wheat germ oil emulsions

Treatment	Storage period (day)					
	0	3	6	9	12	15
M	1.0 \pm 0.013	1.07 \pm 0.023	1.11 \pm 0.012	1.12 \pm 0.014	1.14 \pm 0.023	1.21 \pm 0.013
N	0.9 \pm 0.002	0.97 \pm 0.002	0.99 \pm 0.002	1.05 \pm 0.032	1.11 \pm 0.022	1.17 \pm 0.012

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

it was elicited that, there were moderate differences in TBARs value between micro and nanoWGO emulsions at zero time. On the other hand, nano WGO emulsion (ultrasonication) had lower TBARs value (0.84 mg MDA/ L). Finally, TBARs value was increased from 0.84 to 0.91 mg MDA/L in nano WGO emulsion with low differences; 0.87 to 1.01 mg MDA/ L in micro WGO emulsion at the end of storage at 55°C. It is obvious that, nano WGO emulsion technique permits to characterize it to prevent this formation.

pH value: Due to presence of free fatty acids in the micro and nano WGO emulsions, it was necessary to determine the pH of the oil since it tells the state of oil as to whether the dissociation has taken place or not. The pH of micro and nano WGO emulsions is summarized in Table 4. As seen in the Table, nano WGO emulsion (ultrasonication) recorded pH value 6.46. It could be observed that, nano technique was reported to have a slightly lower acidity than micro technique. Referring to Table (4), during storage period, pH value was gradually decreased as the storage time increasing from 0 to 15days in all emulsions.

The highest reduction rate in pH value was observed with micro WGO emulsion, whereas it was 6.37, 6.29 and 6.27 at 3, 9 and 15 days of storage period.

Acid value (AV): Data in Table (5), illustrated the Acid value (mg /g) of micro and nano wheat germ oil emulsions. Results revealed that, micro WGO emulsion had the highest Acid value of 1mg/g oil, while Acid value in nano WGO emulsion (ultrasonication) was 0.9 mg/g oil. Recorded data showed that, there were a low differences among the nano WGO emulsions at zero time and 6 days as shown with their values (0.90 and 0.99 mg/g oil) but increase was shown between the nano WGO emulsion at zero time and the stored samples after 9,12 and 15 days. Generally, Acid values indicated the low activity of hydrolytic and oxidative enzymes in the micro and nano WGO had occurred. Free fatty acids may be produced by the oxidation of double bonds of unsaturated fatty acid esters. In advanced stages of oxidation, free fatty acids with low molecular weight were developed through the accumulation of acidic products and subsequently increased the acid value [33].

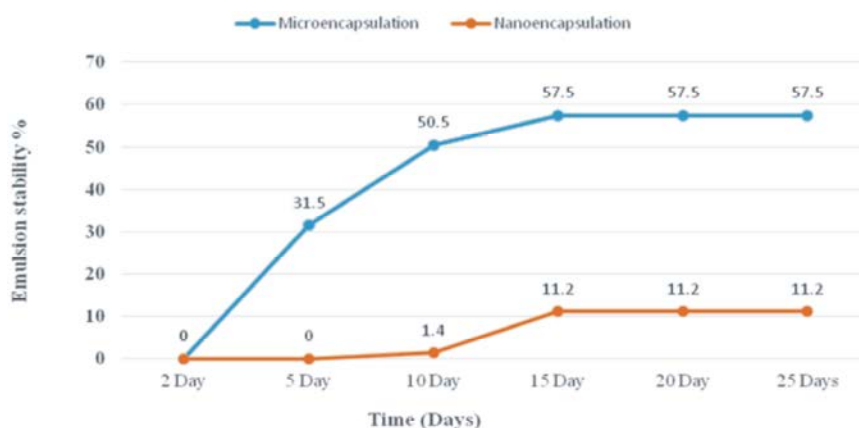


Fig. 1: Emulsion Stability Index (%) of Micro and nano wheat germ oil emulsions

Table 6: Zeta potential and Particle size (µm) of micro and nano wheat germ oil emulsions

M		N	
Particle size (µm)	Zeta potential	Particle size (µm)	Zeta potential
694.3±31.7	-30.21±0.81	71.41±27.15	-32.87±0.56

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

Table 7: Viscosity (cP) of micro and nano wheat germ oil emulsions

Treatment	Storage period (day)				
	0	3	7	14	21
M	165±4.305	165±3.066	162±3.309	160±4.534	160±2.176
N	130±4.016	112±3.052	105±2.820	100±1.043	99±1.981

M Micro wheat germ oil emulsion N Nano wheat germ oil emulsion

Emulsion Stability Index (ESI): The stability of emulsion is very important for various industrial processes. It is very difficult to maintain the stability of an emulsion [34]. Figure (1) illustrates, Emulsion Stability Index (%) of micro and nano WGO emulsions. Obtained data showed that, the ESI of fresh micro and nano WGO emulsions was zero. The same Fig. also showed that, ESI increases by increasing the storage periods. This increase could be due to the occurrence of the sedimentation and then coalescence processes with storage time. The shelf life of such emulsion could not maintain and hence the emulsion might separate. Micro technique highly ESI increased among these values during storage periods compared to zero time revealing the ESI of 31.5, 50.5 and 57.5% from 5th day up to 15days respectively. It could be observed that, after 15 days the two layers were started to separate from each other, later. The main cause of separation of two layers was the separation of water layer which measures the emulsion stability [35].

Particle Size and Zeta Potential: Particle size analysis has been performed in different emulsion formulations, before the drying process. Particle size of emulsions is an

important aspect for retention of core material within the capsules [7]. Table (6) clearly showed that nano technique had a clear effect on decreasing the particle size. Also it can be seen from Fig. (2), While the majority of the particles was 71.41µm for nano WGO emulsion (ultrasonicated sample), for the sample which was homogenized only (micro WGO emulsion), was 694.3µm. Based on these results, it can be said that nano technique (ultrasonication) has a positive impact on emulsification. The electrical charge (i.e. zeta potential) of emulsions can be one of the important parameters that determine the stability of emulsions. Regularly, recorded data of the same Table illustrated micro WGO emulsion had the highest zeta potential of -30.21, while zeta potential in nano WGO emulsion (ultrasonication) was -32.87. Zeta potential could be increased in its negative charge due to some hydrolyzed fatty acids retained at the interface [34].

Viscosity: From the results in Table (7), it could be noticed that, the viscosity value (cP) of micro WGO emulsion was 165 cP which decreased to 130 cP for nano WGO emulsion at zero time. Data of the same Table reported that, at the end of storage period there were high

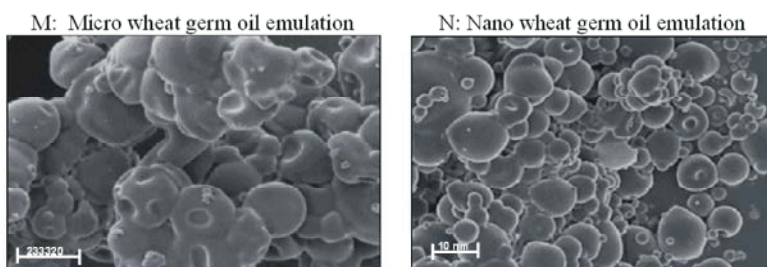


Fig. 2: Transmission Electron Microscopy image of micro and nano wheat germ oil emulsions

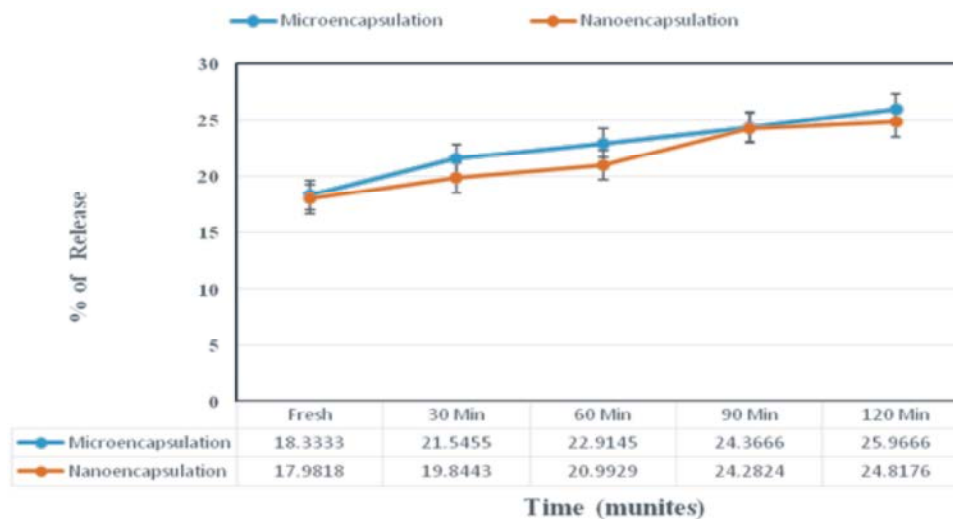


Fig. 3: *In vitro* Digestion (FFAs released %) of micro and nano wheat germ oil capsules

Table 8: Encapsulation Efficiency (%) of micro and Nano wheat germ oil capsules

Treatment	Storage period (day)					
	0	5	10	15	20	25
M	99.30	95.85	93.60	91.00	90.90	86.65
N	100	100	99.35	97.55	96.30	94.25

M Micro wheat germ oil capsules N Nano wheat germ oil capsules

differences among all emulsions which treated with (ultrasonication) or emulsions which treated with (ultraturax homogenization) revealing the values of (99 and 160cP) respectively. Nano WGO emulsion revealed a high decrease in viscosity values giving the average values of 130 cP at zero time compared to 112, 105 and 99 cP at 3, 7 and 21 days respectively.

Transmission Electron Microscopy (TEM): Figure 2 showed Transmission Electron Microscopy image of micro and nano WGO emulsions. The size of nano WGO emulsion was 10 nm. Particles of good quality, which size are less than 40 nm [36]. Characteristics of good nano emulsion were small particle size and low capable of

carrying components [37]. The results of the Transmission electron microscopy image in the present study are in accordance with the Encapsulation Efficiency (%) results (Table 8) where capsules with nano technique (ultrasonication) had higher encapsulation efficiency with less oil left on the surface and more oil trapped inside of the capsules. From the image, it could be noticed that, micro WGO emulsion appear to be made up of spherical particles of about 200 nm in diameter. Characteristics of good micro emulsion were big particle size and high capable of carrying components [38].

Characteristics of Micro and Nano Wheat Germ Oil Capsules

Encapsulation Efficiency (EE): Encapsulation efficiency analysis aims to evaluate the ratio of surface oil to the entrapped oil in the capsule. Encapsulation efficiency is probably the most important criteria for an encapsulation process to be considered as successful [39]. From the data in Table (8), nano WGO capsule had the highest Encapsulation efficiency value by 100%, while Encapsulation efficiency in micro WGO capsule was 99.30%. From the same Table, during storage period,

Encapsulation efficiency values were gradually decreased as the storage time increasing from 0 to 25 days in all emulsions. The highest reduction rate in Encapsulation efficiency was observed with micro WGO capsule, whereas it was 93.60, 90.90 and 86.65 at 10, 20 and 25 days of storage period. Thus, as the viscosity of the micro and nano WGO emulsions decreased (Table7), the encapsulation efficiency values also decreased. This result showed that, ultrasonic homogenization gives better efficiency and quality results in encapsulation of WGO when compared to ultra-turrax homogenization.

In vitro Digestion: The characteristics of capsules type, composition and concentration are some of the key factors that affect the digestion of capsules during in vitro digestion [21]. *In Vitro* Digestion (FFAs released %) of micro and nano wheat germ oil capsules were shown in Figure 3. It could be indicated that, micro WGO capsules had higher FFAs released (18.33%) followed by nano WGO capsules (17.98%) at zero time. From the same figure, data proved that, during the digestion process time, FFAs released values were progressively increased for all capsules under study as the digestion process time increasing from 0 to 120 min. At the end of digestion process time, FFAs released values of all capsules were increased. FFAs released in the WGO capsules ranged between 24.82% for nano (ultrasonic homogenization) and 25.97% for micro (ultra-turrax homogenization).

CONCLUSION

Wheat germ oil (WGO) has been a good source of polyunsaturated fatty acids such as oleic and linoleic acids which have health benefits. It is a highly prone to oxidation acids. Ultrasonic homogenization gives better efficiency and quality results in capsulation of WGO.

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