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Nanoemulsion of Sesame Seeds Oil: Preparation, Evaluation and Stability

A.A. El-Refai[1], M.M. Rabie[1], Rania E. El-Gammal[1] and W.A. Al-Saban[2,*]

[1]Department of Food Industries, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
[2]Department of Food Sciences and Technology, Faculty of Environmental Sciences and Marine Biology, Hadramout University, Hadhramaut, Yemen

*Corresponding author: E-mail: wailalsabban2016@gmail.com

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This work aims to study the effect of using nanoemulsion particles on the stability of sesame seed oil emulsion. During the roasting process of sesame seeds (Sohage-1), some physico-chemical properties of sesame seeds and its oil were determined. The moisture, protein and fiber contents were decreased whereas oil, ash and carbohydrates contents were increased. Sesame oil was extracted from roasted sesame seeds using mechanical pressing; some physico-chemical properties for extracted oil were determined. Results indicated that colour, acid value, free fatty acids (%), peroxide and hydrolysis values were increased, while saponification value was decreased in sesame oil. Also traditional and nanoemulsions form of sesame oil were prepared.Characterization of these emulsions particles was conducted using zeta potential and transmission electron microscope (TEM). The results also indicated that small droplet size of nanoemulsion particles being 26.28 nm in emulsion which prepared by using nano-technique as compared with those of 638.8 nm in traditional emulsion type and low polydispersity index (PDI) was nanoemulsion particles 0.266. This lead to more uniformity in droplet size thus could improve the stability of emulsion system. The TEM results of nanoemulsion particles of sesame oil showed that spherical droplets and nearly similarity in shape in nanoemulsion in compared with semi-spherical and varied particles size in traditional one. Creaming index, centrifugation test, conductivity and freeze-thaw cycles were used to evaluate all type of prepared emulsion stability. The nanoemulsion particles of sesame oil were found to be more stable than those of traditional one. These results indicated that the nanoemulsion process could increase the stability of prepared emulsion. Thus, using of nanoemulsion technique could be used as commercial way to enhance the stability of prepared emulsion.

Keywords: Emulsion, Nanoemulsion, Sesame oil.

INTRODUCTION

Sesame seeds (*Sesamum indicum* L.) belong to the *Tubiflorae* order and *Pedaliaceae* family [1]. Known as one of the World's most significant oil seed crops contains nearly about 40 to 60 % oil [2]. Several reviews [3-5] are reported regarding the properties and its nutritional values.

Emulsion is characterized as a heterogeneous system, comprising in any event two immiscible stages, one of them is scattered as droplets (scattered stage or inner stage) while the other fluid stage in particular (continuous stage or outer stage). They are alluded W/O emulsions, water droplets scattered in an oil medium or O/W emulsions, oil drops scattered in a fluid medium or emulsions are scatterings comprised of two immiscible fluid stages which are blended utilizing mechanical shear and surfactants. Molecule size of this ordinary emulsion grows persistently with time and partition happens at gravitational power consequently these emulsions are thermodynamically unstable [6].

Emulsion stability indicates an ability of an emulsion to oppose changes in its properties after some time: progressively stable emulsion, the more gradually its properties changes [7]. The apparent quality of emulsion dependent on food products which are strongly affected by their stability, rheology and appearance [8]. Principle markers of losing stability is due an increase in droplet mean width of emulsion and the development rate of droplets can uncover the component mindful. Emulsion stability is profoundly affected by specific gravity, droplet size and its distribution, and rheological properties. Adding the hydrocolloids to the watery stage can yield specific rheological properties to accomplish emulsion dependability. A few hydrocolloids go about as a surface dynamic gums being able to shape a film around the oil droplets [9].

Nanoemulsions are scatterings of nano-scale droplets framed by shear-instigated bursting. Nanoemulsions are characterized as W/O (water-in-oil) or O/W (oil-in-water) emulsion creating a transparent product that has a droplets measure between 20 and 200 nm and does not have the penchant to combine. Nanoemulsions have several fascinating physical characteristics that are not quite the same as or are more extraordinary than those of small scale emulsions. Nanoemulsions show up different from microscale emulsions since the droplets can be a lot littler than optical wavelengths visible. Therefore nanoemulsions can show up about transparent in the visibly and display a little dispersions [10].

Ghosh *et al*. [11] noted that oil type, surfactant type, surfactant concentration, blending proportion of oil-surfactant and sonication time indicated important effect on droplet size of nanoemulsion. Accordingly, the investigation was a trial to prepare sesame oil nanoemulsion using ultrasonic. The quality attributes of obtained nanoemulsion was evaluated compared with the traditional sesame emulsion. Also, the stability of sesame oil nanoemulsion was studied.

EXPERIMENTAL

Seeds of sesame (*Sesamum indicum* L.) namely; Sohage 1 was purchased from Oil Seeds Department, Field Crops Institute Research, Agricultural Research, Center Giza, Egypt.

All chemicals, solvents and Tween 80 (polyoxyethylene sorbitan monooleate (polysorbate 80)) were purchased from El-Gomhouria medications Company, Cairo, Egypt.

Preparation of sesame seeds: Sesame seeds were cleaned manually by washing all foreign matters and then divided into three different parts as follows:

(a) **Oven roasted sesame seeds:** The seeds of sesame were roasted at 200 ºC for 15 min using a heating oven (ED-115, Serial No 12-18171, Germany) as reported by Hassan [12].

(b) **Vacuum roasted sesame seeds:** The seeds of sesame were roasted at 65 ºC for 15 m using an electric oven under vacuum (Thermo Scientific, model 6255, USA).

(c) Last sesame seeds part without any thermal treatment as control one.

Extraction of sesame oil: Sesame seeds (Sohage1) were extracted using mechanical pressing and then stored at 4 ºC until further analysis was carried out.

Gross chemical composition: Moisture, crude protein, crude oil, crude fiber and ash contents were estimated according to AOAC method [13]. Carbohydrates content was calculated by the difference as reported by Pellet and Sossy [14]. Total energy value (kcal/100 g) was estimated according to Ekanayak *et al*. [15].

Physical properties of sesame oil: Refractive index and colour were determined according to O'Keefe and Pike [16] while specific gravity was estimated according to AOAC method [13].

Chemical properties of sesame oil: Free fatty acids, acid, iodine, saponification and peroxide values were estimated according to O'Keefe and Pike [16]. While hydrolysis value was calculated from acid value and saponification value according to the methods described by Ibrahim *et al*. [17].

Preparation of sesame oil emulsions: Emulsion was prepared using sesame oil from sesame seeds variety (Sohage-1), addition of surfactant (Tween 80) and distilled water in the ratio of 10:5:85. Then sesame oil was mixed with Tween 80, and the mixture added to distilled water at 75 ºC using magnetic stirrer having 1100 revolutions per minute (rpm) for 5 min. The emulsion was then divided into two parts :

(a) First part (traditional) were mixed using magnetic stirrer for 40 min to prepare traditional emulsion type.

(b) Second part was mixed using microscale droplets and sonicated for 45 min at 90 % amplitude at frequency of 20 KHz and power of 500 W to prepare nanoemulsion as reported by Rocha-Filho *et al*. [18] and Sarojini *et al*. [19].

Droplet size and size distribution: Droplet size samples distribution and polydispersity index (PDI) were measured using particle size analyzer.

Morphology and structures: Morphology and structure of emulsion and nanoemulsion were examined utilizing transmission electron microscopy (TEM) (JEM-2100, JEOL) working at 70 kV equipped for point-to-point resolution. Blend of brilliant field imaging at expanding amplification and diffraction modes was utilized to uncover the structure and size of the emulsion and nanoemulsion. A drop of emulsion and nanoemulsion was reasonably weakened with water and applied on a carbon-coated grid. The coated grid was dried and after that taken on a slide and secured with a spread slip and saw under the microscope [20].

Stability of sesame oil emulsions

Creaming index: Emulsions samples (10 g) were transferred to closed tight tubes and stored at 25 ºC for 24 h. Oil droplets have a lower density than water phase and thus appears at the upper layer during storage. The height of total emulsifier (HE) and height of depleted bottom layer (HD) was measured [21].

Centrifugation test: Emulsions samples (15 mL) underwent a centrifugal acceleration at 3,500 rpm for 30 min at 25 ºC. After that, phase separation was observed as reported by Rocha-Filho *et al*. [18].

Freeze-Thaw cycles: Samples of emulsion were stored in a tightly sealed tube vertically for 24 h in a freezer at -5 ºC and then for 24 h at room temperature (25 ºC). The samples were observed, and changes were recorded. The cycle was repeated for three times as mentioned by Silva *et al*. [22].

Conductivity: Conductivity was measured using conductivity meter (JENCO, 3173, conD, USA) at room temperature.

Statistical analysis: Obtained data were analyzed with analysis of variance (ANOVA) procedures by using Co-state statistical software. Differences between means compared by LSD at 5 % level of significant [23].

RESULTS AND DISCUSSION

Effect of roasting of sesame seeds

Gross chemical composition: Gross chemical composition of untreated and roasted sesame seeds are given in Table-1. Moisture content was gradually decreased from 5.68 to 6.02 % for untreated seeds to 5.28 % and 3.56 % for vacuum and oven roasted seeds, respectively. Results obtained that there were significant differences at $p < 0.05$ in moisture content of sesame seeds (Sohage-1) and ranged between 3.56 and 6.04 %.

TABLE-1
EFFECT OF ROASTING PROCESS ON GROSS CHEMICAL COMPOSITION OF UNTREATED AND ROASTED SESAME SEEDS (SOHAGE-1)

Constituents (%)	Treatments		
	Untreated	Roasted	
		Vacuum	Oven
Moisture	6.02^{a}	5.28^{c}	3.56^{f}
Protein	21.89^{a}	21.79^{ab}	21.24^{b}
Oil	43.95^{e}	48.72^{c}	50.97^{a}
Ash	3.84^{d}	4.59^{c}	5.07^{b}
Crude fiber	17.52^{b}	11.92^{d}	11.46^{e}
Total carbohydrates	6.78^{c}	7.70^{b}	7.70^{b}
Energy (kcal/100 g)	510.22^{e}	556.46^{c}	574.49^{a}

Means of triplicate samples. a, b, c, d, f: means having different superscripts within the row are significantly different at $p < 0.05$.

Simiarly, results showed that protein content was 21.89 % for untreated seeds of Sohage-1, while decreased to 21.24 % in oven roasted Sohage-1. It also indicated that there were significant differences at $p < 0.05$ in protein content between untreated seeds and oven roasted of sesame seeds (Sohage-1), while no significant difference was observed in protein content between untreated and vacuum roasted seeds. The decrease in protein content may be due to the roasting process accelerates the Maillard reactions and subsequently making the protein and its amino acids significantly unavailable for digestion.

Oil contents were also found to be increased in untreated oil of Sohage-1 from 43.95 to 48.72 % and 50.97% for vacuum and oven roasted seeds, respectively. There were significant differences at $p < 0.05$ in oil contents of sesame seeds. An increase in oil content of roasted sesame seeds may due to loss of moisture during roasting. Crude proteins and the high proportion of oil give this seed a featured potential for the oil industry.

As shown in Table-1, vacuum roasting of Sohage-1 seeds increased in the amount of ash content to 4.59 % in compared with 3.84 % for untreated Sohage-1 seeds. Oven roasted seeds of Sohage-1 increased the amount of ash and total carbohydrates to 5.07 % and 7.70 % as compared to 3.84 % and 6.78 % for untreated Sohage-1 seeds, respectively. There were significant differences at ($p < 0.05$) in ash, fiber and total carbohydrates contents of sesame seeds (Sohage-1). The obtained result also indicated a decrease in crude fiber from 17.52 % for untreated seed to 11.92 % and 11.46 % in both of vacuum and oven roasted seeds. In present work, some chemical composition of sesame seeds were varied which may be due to the variety, size, origin and colour of the seeds. However, these results are similar to those of Hassan's work [12].

Physico-chemical properties of sesame oil: Effect of roasting process on physico-chemical properties of sesame oil are presented in Table-2. Untreated and roasted sesame oil nearly had the same refractive index and specific gravity values while results showed that there were significant differences at ($p < 0.05$) in colour of oil samples. Vacuum and oven roasted sesame seeds were increased in colour to 2.92 and 3.12 as compared to 1.39 for untreated sesame seeds. A significant was observed at $p < 0.05$ in specific gravity in both of untreated and roasted sesame seeds oil.

There were significant differences at $p < 0.05$ in free fatty acids (%), acid value and peroxide value of untreated and roasted sesame oil. So acid value is an indication of amount of fatty acid present in the oil samples. Acid value was 3.33 mg KOH/g oil in untreated oil, while acid value increased to 4.56 and 5.20 mg KOH/g oil for vacuum and oven roasted oil.

TABLE-2
EFFECT OF ROASTING PROCESS ON PHYSICOCHEMICAL PROPERTIES OF UNTREATED AND ROASTED SESAME SEED OILS

Properties	Treatments		
	Raw	Roasted	
		Vacuum	Oven
Refractive index at 25 °C	1.474^{b}	1.474^{b}	1.475^{a}
Photometric color index	1.39^{e}	2.92^{b}	3.12^{a}
Specific gravity	0.954^{a}	0.942^{b}	0.940^{c}
Acid value (mg KOH/g oil)	3.33^{c}	4.56^{b}	5.20^{a}
Free fatty acids (as oleic acid %)	1.67^{c}	2.29^{b}	2.61^{a}
Saponification value (mg KOH/g oil)	187.18^{b}	180.31^{e}	188.18^{a}
Iodine value (Ig/100 g oil)	106.99^{c}	109.32^{abc}	107.30^{c}
Peroxide value (Meq O_2/kg oil)	4.62^{f}	5.07^{d}	10.01^{a}
Hydrolysis value	1.78^{c}	2.53^{b}	2.76^{a}

Means of triplicate samples. a, b, c, d, f: means having different superscripts within the row are significantly different at $p < 0.05$.

The peroxide value measures the content of hydroperoxides in the oil this an index of rancidity, so the high peroxide value of oil referred a poor resistance of the oil to peroxidation. Results showed that the peroxide value in vacuum and oven roasted oil increased to 5.07 and 10.01 meq O_2/kg oil compared with that of untreated oil samples (4.62 meq O_2/kg oil). Tuned-Akintunde *et al.* [24] showed that the peroxide value varied between 2.22 and 15.07 meq O_2/kg oil for local sesame oil and 2.24-10 meq O_2/kg oil for improved one.

Results also indicated that there were significant differences at $p < 0.05$ in saponification and hydrolysis values of untreated and roasted oil samples. So saponification is an indicator of average molecular weight (or chain length) of all the fatty acids present. A decreased in saponification value in vacuum roasted oil to 180.31 mg KOH/g oil while that of oven roasted oil was increased to 188.18 mg KOH/g oil in compared with that of untreated oil samples(187.18 mg KOH/g oil). Tunde-Akintude *et al.* [24] also reported that saponification value of local sesame oil was varied between 174 and 196.32 mg KOH/g oil and between 182.31 and 198.02 mg KOH/g oil for improved cultivars.

Low iodine value oil contains a lower number of double bonds than high iodine value oil and usually has increased oxidative stability. In the present work, no significant difference in iodine value in sesame seeds oil samples is found. The obtained results are in agreement with those of El-Khier *et al.* [25] and Tunde-Akintude *et al.* [24].

Droplet size and size distribution: Droplet size and size distribution of emulsion and nanoemulsion of sesame oil are shown in Fig. 1. Means of droplet size were 638.8 and 26.25 nm in emulsion and nanoemulsion of sesame oil, respectively. While polydispersity index (PDI) values were 1.00 and 0.266 in emulsion and nanoemulsion of sesame oil, respectively. A decrease in droplet size of nanoemulsion may be attributed to ultrasonic waves which used of preparing shear to micro-scale droplets and altering them to nano-scale [26]. The lower the PDI, the higher the uniformity of droplet size in formulation,

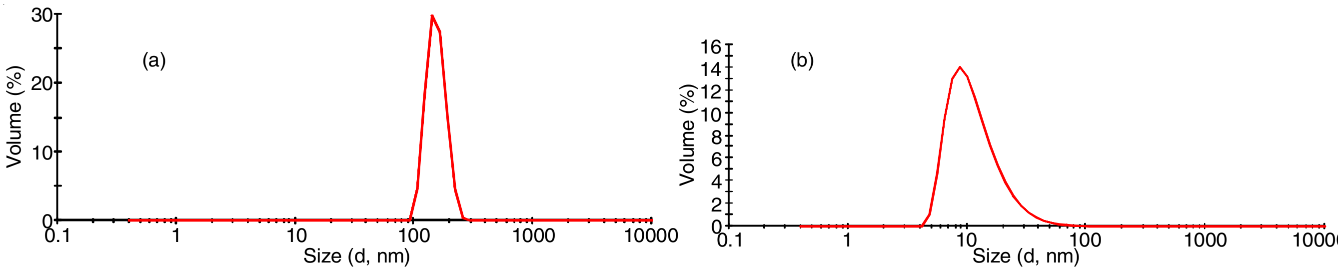

Fig. 1. Droplet size and size distribution of traditional emulsion (a) and nanoemulsion (b) of sesame oil

this means polydispersity index (PDI) measures the homogeny and satbility of the droplet size in the emulsion [19,27]. So increased in polydispersity index (PDI) to 1.00 in emulsion may be lead to lower the uniformity of droplet size in emulsion on contrary in nanoemulsion of sesame oil.

Morphology and structure droplets: Morphology of emulsion and nanoemulsion were visualized by transmission electron microscopy (TEM) are shown in Fig. 2. The emulsion droplets were semi-spherical in morphology and particles size were varied between small and large and the average droplet size was found to be 300 nm. While nanoemulsion particles has as spherical droplets and particle size nearly the same in size and the droplet size were also found to be 62.2 nm. It is found that all traditional emulsion droplet were varied in TEM micrograph whereas the other prepared by nanoemulsion techniques were nearly similar this results may be to the aggregation process using ultra sonicator.

Stability indices of traditional emulsion and nanoemulsion of sesame oil: Table-3 showed that the nanoemulsion sesame seed oil was more stable than the other traditional emulsion and good stability value may be because of sonicator for cracking the emulsifying particles into small parts, which leads to the mixing the particles to form homogenous and therefore high stability of nanoemulsion particles size. Results also showed that no separation phase were seen after centrifugation and Freeze-Thaw cycles, which further confirmed that this nanoemulsion particles are stable.

TABLE-3
STABILITY INDICES OF TRADITIONAL EMULSION AND NANOEMULSION OF SESAME OIL

Stability indices	Traditional emulsion	Nanoemulsion
Creaming index	1.33	Stable
Centrifugation test	Stable	Stable
Freeze-Thaw cycles	Stable	Stable
pH value	5.91	6.04
Conductivity (mS/cm)	0.08	0.09

Monitoring the pH value is significant for deciding the emulsions stability due to changes in pH indicated the event of chemical reactions that can be compromised [28]. The pH value were found to be 5.91 and 6.04 for traditional emulsion and nanoemulsion.

Conductivity of the nanoemulsions was estimated to determine the stage system (W/O or O/W) of nanoemulsion [29]. Obtained resulted of conductivity were 0.08 and 0.09 in traditional emulsion and nanoemulsion might be indicated to that water-in-oil nanoemulsion, where water is in the inside stage is less conducting. Furthermore, oil-in-water nanoemulsions are highly conducting due to water is in the outside stage [30]. From the conductivity results in Table-3, the emulsion and nanoemulsion could be distinguished as oil-in-water nanoemulsion.

Conclusion

In conclusion, the results obtained indicated that chemical composition of sesame seeds and physico-chemical properties

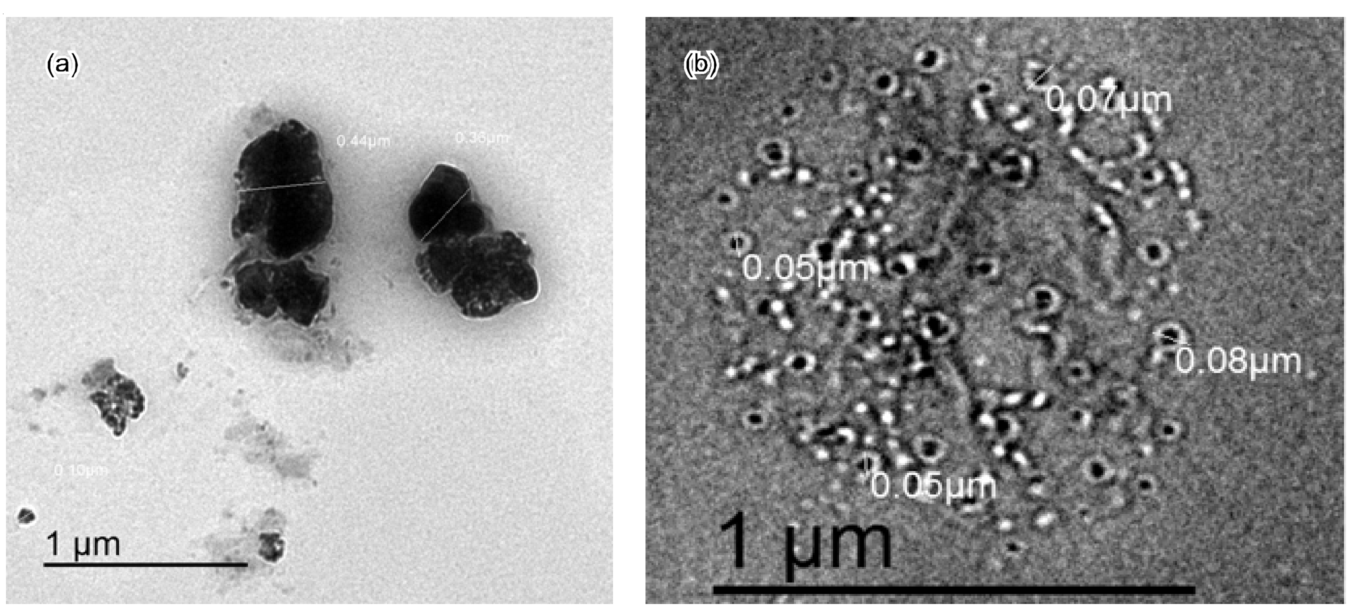

Fig. 2. TEM analysis of obtained traditional emulsion (a) and nanoemulsion (b) particles of sesame oil

of sesame oil were affected during roasting. Results also showed that low droplet size and low poly dispersity index (PDI) of nanoemulsion particles lead to more uniformity in droplet size, thus improve the stability of emulsion system. Finally, it can be concluded that nanoemulsion process could increase the stability of prepared emulsion.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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