Asian Journal of Scientific Research

ISSN 1992-1454

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OPEN ACCESS Asian Journal of Scientific Research

ISSN 1992-1454
DOI: 10.3923/ajsr.2019.516.523

Research Article
Microencapsulation of Date Seed Oil by Spray-drying for Stabilization of Olive Oil as a Functional Food

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Abstract

Background and Objective: Microencapsulation of oils in spray dried powder is an effective approach to protect against oxidation. This study investigated the influence of microencapsulated date seed oil on the stability of olive oil. **Materials and Methods:** Date seed oil was extracted from two *Phoenix dactylifera* L. seed varieties (Ramly and Helwa Aljouf) and the saponification (SV), acid (AV), iodine (IV) and peroxide (PV) values and the fatty acid profile were determined. Microencapsulation of date seed oil was carried out in gum Arabic and maltodextrin (1:1 w/w) using a spray dryer. The date seed oil powder was characterized and the total phenolic content, radical scavenging activity (RSA) and oxidative stability of olive oil after addition of microencapsulated date seed oil were measured. **Results:** Ramly and Helwa Aljouf seed oils showed AV of 0.87 and 0.41 mg KOH g^{-1} oil, PV of 3.42 and 1.02 meq O_2 g^{-1} oil, SV of 199.05 and 204.15 mg KOH g^{-1} oil and the IV was 75.51 and 54.86 g I_2/100 g oil, respectively. The fatty acid profile showed 17.13% linoleic acid (C18:2) and 2.3% linolenic acid (C18:3) in Ramly date seed oil, whereas the Helwa variety contained 6.73% linoleic acid and no linolenic acid. Helwa Aljouf date seed oil contained 55% total phenols more than the Ramly oil and showed a higher RSA. In addition, Helwa Aljouf, Ramly and the encapsulated Helwa Aljouf seed oils enhanced the oxidative stability of olive oil. **Conclusion:** Helwa Aljouf and Ramly seed oils showed higher oxidative stability when compared with olive oil and both oils increased the oxidative stability of olive oil. In addition, the microencapsulated date seed oil provided additional protection and improved the nutritional value and functionality of olive oil.

Key words: Microencapsulation, *Phoenix dactylifera*, olive oil, date seed oil, antioxidant stability, spray-drying, Ramly, Helwa Aljouf

Citation: Khaled El-massry, Ahmed El-Ghorab, Amr Farouk, Said Hamed, Hassan Elgebaly, Nermeen Mosa, Ibrahim Alsohaimi and Ayman Mahmoud, 2019. Microencapsulation of date seed oil by spray-drying for stabilization of olive oil as a functional food. Asian J. Sci. Res., 12: 516-523.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There is a global concern about the use of food processing by-products that could be used to produce value-added products. Peels and seeds are rich in precious nutrients with properties that could be improved and incorporated into human diet. In Saudi Arabia, the date seeds constitute 10-15% of the fruit mass production. These by-products contain dietary fibers, crude oil (10%), fatty acids (FAs), proteins, minerals, sugars, antioxidants, phytosterols and polyphenols[1]. To improve the product availability and cover the emerging needs for new oil sources, waste has been incorporated into the high-quality production of special oils, food, pharmaceutical, chemicals or cosmetic industries. Therefore, new sources of oils rich in phytosterols and phenolic compounds and have lipid profile similar to the conventionally consumed oils are needed[2].

Given their rich content of bioactive compounds, such as essential FAs, phytosterols, tocopherols, phenolic compounds and carotenoids, fruit seeds-derived oils can represent alternative sources of oils with efficient properties[3]. Date seeds contain 54.0-70.0% linoleic acid (ω-6) and 15.0-24.0% oleic acid (ω-9); therefore, possess a great nutritional value[4]. Date seeds have 14 types of saturated and unsaturated FAs, including palmitoleic, oleic, linoleic and linolenic acids[5]. The nutritional and medicinal activities of date seeds, such as anti-inflammatory, gastro protective, antiviral, gonadotropic and nephroprotective efficacies were related to their chemical composition[6]. Date seed oil showed a protective effect against hepatic dysfunction in diabetic animals and chemically-induced oxidative stress in normal human skin[7,8]. In addition, date seeds have shown other applications in food, fermentation, chemical and feed industries[6]. Date seed extract is a rich source of polyphenols[4,9] which are known to have antioxidant activity[10]. However, these polyphenols are responsible for some unfavorite sensory characteristics associated with food quality, such as the bitter taste and undesired color[11]. Furthermore, polyphenols showed low permeability and solubility in gut and instability under stress conditions encountered in foods[12]. Encapsulation of these ingredients may overcome these drawbacks and widen their utilization in food products[13].

Olive oil is a functional food with dietetic importance and therapeutic characteristics[14]. It contains valuable constituents, such as unsaturated FAs, medium-chain FAs, carotenoids, squalene and vitamins, which are susceptible to oxidation upon prolonged exposure to oxygen, light, elevated temperature and moisture[15]. Oxidation can damage the nutrients and flavors transformation of unsaturated to saturated FAs and generation of harmful reactive oxygen species. Hence, inhibition of lipid oxidation is critical for improving the quality of olive oil[16].

Microencapsulation is the incorporation of a functional core substance within a shell of a wall material for conferring protection and ease of handling. Different methods were developed to fulfill an efficient encapsulation. These include spray drying, an economically feasible method commonly used for the microencapsulation of ingredients in powder form for food applications[17]. To date, nothing has yet been reported on the effect of microencapsulated date seeds oil on the oxidative stability of olive oil. However, this approach was investigated for other plants oils, including turmeric[18] and cardamom[19]. Therefore, this study was conducted to test the hypothesis that the addition of encapsulated date seed biomolecules can fortify olive oil to be used as a functional food.

MATERIALS AND METHODS

This study was conducted during the period from January, 2018 to February, 2019. Two *Phoenix dactylifera* L. seed varieties, Helwa Aljouf and Ramly, were purchased from local markets in Sakaka (Saudi Arabia) and Egypt, respectively. Olive oil, without any added antioxidants, was supplied by the National Research Centre (NRC, Giza, Egypt). Gum Arabic (GA) and maltodextrin De15 (MD) were supplied by Edwic Chemical Company (Egypt) and PPZ NOWAMYL (Lobez, Poland), respectively. Butylated hydroxyanisole (BHA), Tween 80, n-pentane, n-hexane and diethyl ether were purchased from Sigma (St. Louis USA).

Extract preparation: The seeds of Helwa Aljouf and Ramly dates were washed to remove any adhering flesh, air dried followed by further drying for 5 h at 50°C. The seeds were ground (1-2 mm) and then kept at 4°C.

Soxhlet extraction method: A total of 25 g date seeds powder was dissolved in 400 mL n-hexane in a Soxhlet extractor for 6 h. The extracts were evaporated by rotary evaporator and seed oils were drained under a stream of nitrogen and then stored at -20°C until used.

Hexane extraction method: About 100 g of the date seed powder was homogenized in 500 mL hexane and mixed for 4 h by shaking at 180 U min^{-1}. After centrifugation at 1000 g for 15 min, the supernatant was collected and filtered using Whatman No. 2 filter paper.

Determination of the physicochemical characteristics of date seed oil: Saponification value (SV), acid value (AV), iodine value (IV) and peroxide value (PV) of the extracted date seed oils were determined using standard IUPAC methods for the analysis of fats and oils[20].

Date seed oil emulsion formulation: The emulsion was formed by hydrating 15 g GA in 100 mL deionized water overnight at 4°C. About 15 g MD was dissolved in the gum solution and 1% Tween 80 was added followed by the addition of Helwa seed oil (HO). The whole mixture was magnetically stirred for 5 min and homogenized for 20 min at 200.0 W, 24 kHz using UP200S ultrasound homogenizer (IKA Hielscher GmbH, Berlin, Germany) equipped with an impulse generator. The emulsion vessel was immersed in a cold-water bath during the whole process[21].

Spray drying procedure: The date seed oil emulsion was spray dried using a Mini Spray Dryer (B-290, BÜCHI, Switzerland) as previously described[22]. To get two different batches, the drying procedure was conducted twice.

Characterization of the Helwa oil (HO) powder

Moisture content: The moisture percentage of HO powder was calculated on a wet basis by heating 10 g of the HO powder in the Petri dish at 105°C till a persistent weight was attained.

Bulk density: The bulk density of encapsulated oil was determined by the tapping method[23]. Two grams of the HO powder was loosely weighed and added into a 10.0 mL graduated cylinder. The cylinder containing the powder was tapped on a flat surface to a constant volume. The bulk density was calculated by dividing the sample weight by its constant final volume.

Powder morphology: The particles morphology of the HO powder was analyzed using field emission scanning electron microscope (SEM; Quanta FEG 250, FEI, Czech Republic) at accelerating voltage 20 kV. The powder was mounted on aluminum stubs with double sided adhesive tape and coated with gold using an Edwards sputter coater S150A (Crawley, England).

Particle size distribution: The HO powder size distribution was studied by the static light scattering instrument (Mastersizer Hydro 2000, Malvern Instruments, UK). Samples were measured in triplicates.

Determination of surface HO by solvent extraction at room temperature: The surface HO powder was examined as previously described[24] with few modifications. Five g of the HO concentrate was dripped in 75 mL n-pentane and the mixture was continuously twirled to support HO powder washing from the surface. The solvent was filtered and vacuum evaporated at 40°C using slow stream of nitrogen. The extractable surface HO was calculated (% weighed).

Determination of encapsulation efficiency of HO: The encapsulation efficiency of HO was calculated according to the following equation:

$$EE_{HO} = \frac{W_{THO} - W_{SHO}}{W_{THO}} \times 100$$

Where:

EE_{HO} = HO encapsulation efficiency

W_{THO} = Total calculated weight of HO in a sample based on 1:4 w/w of HO powder in the initial formulated emulsion

W_{SHO} = Extractable surface HO weight practically found in the same weight of powder

Determination of fatty acid composition: A total of 100 mg of the two date seed oil samples was converted to methyl esters[25]. One microliter of fatty acid methyl esters of the tested samples was investigated using gas chromatography (Hewlett-Packard 5890 Series II GC) equipped with FID and a capillary column (HP Inovax cross-linked PEG, 30 m×0.32 mm×0.25 mm film). The analysis program was: Temperature of column 180-240°C at rate 5°C min^{-1}, the injector and detector temperature were fixed at 250°C. The carrier gas was nitrogen and peaks were identified by comparing the retention times with reference compounds analyzed under the same conditions.

Measurement of total phenolics: The total phenolic content was determined using Folin-Ciocalteu reagent[26] and the results were expressed as mg g^{-1} gallic acid equivalents (GAE).

Measurement of radical scavenging activity (RSA): The RSA was measured using DPPH• scavenging assay[27]. The absorbance was read at 517 nm on a UV-vis mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan).

Determination of oxidation induction time: The oxidative stability was expressed as the oxidation induction time measured with the Rancimat 679 apparatus (Metrohm AG, Herisau, Switzerland). Briefly, 2.5 g oil sample was heated to 100°C and a purified air flow rate of 15 L h^{-1}. The volatile degradation products were trapped in distilled water and determined conductometrically. The induction time was defined as the time necessary to reach the inflection point of the conductivity curve[28].

Statistical analysis: The results were analyzed by means of T-test using GraphPad Prism 5 (GraphPad Software, CA, USA). The data were presented as mean±standard deviation (SD). A $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Physical appearance of the date seed oil: The physico-chemical characteristics of the two extracted seed oils from *Phoenix dactylifera* L. cultivars Ramly (Egypt) and Helwa Aljouf (Saudi Arabia) were represented in Table 1. The two oils were semisolids below10°C and viscous liquids at room temperature.

Ramly and Helwa Aljouf seed oils showed AVs of 0.87 and 0.41 mg KOH g^{-1} oil and PVs of 3.42 and 1.02 meq O_2 g^{-1} oil, respectively (Table 1). These findings suggested that the oil could be kept for a long time without deterioration[15], but the Helwa variety is more stable because of its lower AV and PV when compared with the Ramly variety.

The low AV and PV of the two seed oils supported the idea that they are safe for human consumption[4,29] and for use in foods, cosmetics and pharmaceuticals. The SVs of the Ramly and Helwa seed oils were 199.05 and 204.15 mg KOH g^{-1} oil, respectively (Table 1). These results pointed to their remarkable higher content of low molecular weight triacylglycerols (TAG). These SV values resemble those of canola[30,31], palm[32] and raspberry seed oils[33].

As shown in Table 1, the IVs of Ramly and Helwa Aljouf seed oils were 75.51 and 54.86 g I_2/100 g oil, respectively. These results indicated that Helwa Aljouf seed oil contained less unsaturated FAs than Ramly seed oil. Accordingly, Ramly date seed oil has been previously reported to have an IV of 75.3 g I_2/100 g oil[34] . On the other hand, the IV of other varieties of date seed oils ranged from 44.1 g I_2/100 g in Deglet Nour to 45.5 g I_2/100 g[35]. These values were lower than the obtained IV of Helwa seed oil. This variation could be attributed to differences in varieties, genetics and climatic conditions[36].

The two tested date seed oils showed different content of saturated and unsaturated FAs (Table 1). Oleic acid was the main unsaturated FA in Ramly (39.72%) and Helwa oil (38.5%), while lauric acid represented 17.22 and 20.93% in Ramly and Helwa date seed oils, respectively. Linoleic acid (C18:2) in Ramly date seed oil (17.13%) was higher than its concentration in the Helwa variety (6.73%). The results showed linolenic acid (C18:3) in the Ramly date seed oil, but not in the Helwa variety. These variable FAs in the two date seed oils were reflected by the lower PV of Helwa Aljouf oil (1.02 meq O_2 g^{-1} oil) than that of Ramly (3.42 meq O_2 g^{-1} oil) and also affected the oxidative stability.

Total phenolics content and RSA: As depicted in Fig. 1, Helwa Aljouf date seed oil showed 55% total phenols (34 mg/100 g GAE) higher than the Ramly oil (14 mg/100 g GAE). The higher content of polyphenols seemed to be a major cause of the observed higher RSA of Helwa Aljouf seed oil (72%) when compared to Ramly oil (50%) as represented in Fig. 2. The RSA% is a primary marker compounds' contribution to the total antioxidant potential[37]. Accordingly, the previously reported improvement in oxidative stability of Deglet Nour seed oil over the Allig seed oil was attributed to the higher content of total phenols[35].

Table 1: Physico-chemical characteristics of Ramly and Helwa Aljouf seed oils

Characteristics	Ramly date seed oil	Helwa Aljouf date seed oil
Oil content (% DW*)	4.35	7.80
Acid Value (mg KOH g^{-1} oil)	0.87	0.41
Peroxide Value (meq O_2 kg^{-1} oil)	3.42	1.02
Saponification Value (mg KOH g^{-1} oil)	199.05	204.15
Iodine Value (g I_2/100 g oil)	75.51	54.86
Fatty acid profile		
Lauric (C12:0) (%)	17.22	20.93
Myristic (C14:0) (%)	6.44	12.96
Palmitic acid (C16:0) (%)	10.14	10.94
Stearic acid (C18:0) (%)	7.27	9.94
Oleic acid (C18:1) (%)	39.72	38.50
Linoleic acid (C18:2) (%)	17.13	6.73
α-Linolenic acid (C18:3) (%)	2.23	0.00
SAFA (%)	40.92	54.77
MUFA (%)	39.72	38.50
PUFA (%)	19.36	6.73
Total unsaturated fatty acids (%)	59.08	45.23

*DW: Dry weight basis, SAFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acid

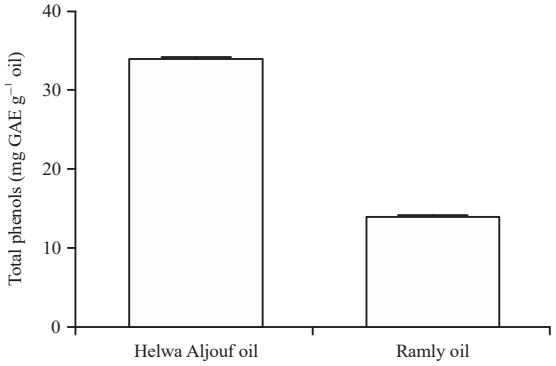

Fig. 1: Total phenolic content of Helwa Aljouf and Ramly seed oils
Data are mean±SD, n = 3

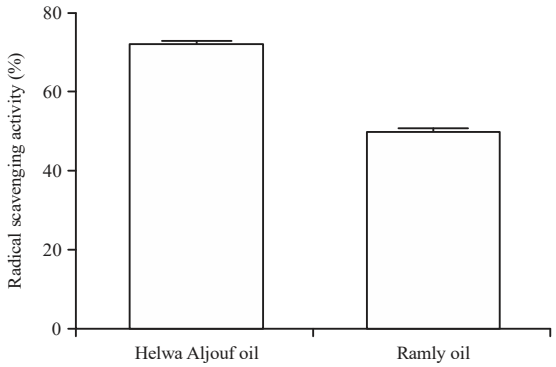

Fig. 2: Radical scavenging activity of Helwa Aljouf and Ramly seed oils
Data are mean±SD, n = 3

Basic parameters of date seed oil powder: The physicochemical characteristics of the date seed powder produced by spray drying were reported. The moisture content was relatively within the range which is under the minimum specification for several powders used in food applications, minimizing the chance of microbial contamination and lipid oxidation[38]. As depicted in Fig. 3a, SEM examination revealed no roughness on capsule surface and such imperfections were formed as a result of slow film formation during the drying of atomized droplets. Additionally, it showed the presence of MD as the main encapsulating agent enabled the formation of more homogeneous capsules with better spherical shape and smoother surface. As represented in Fig. 3b, well-defined spherical microcapsules were observed beside other larger agglomerates of irregular shapes. In addition, small and large particles of oil emulsion were present after reconstitution in water and the surface mean diameter was 1-100 µm (Fig. 4).

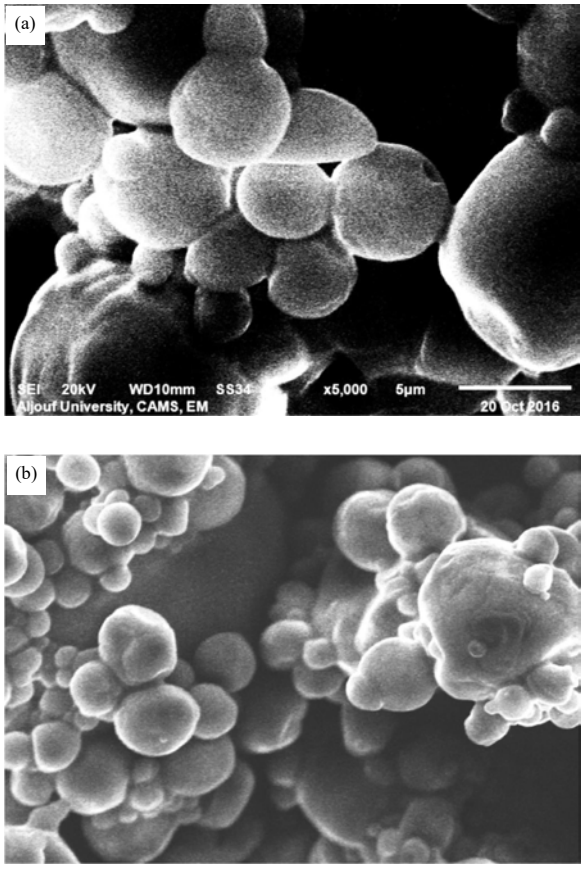

Fig. 3(a-b): SEM of a spray-dried powder at 5 µm. (a) SEM examination offered no roughness on capsule surface and (b) Discrete sphere-shaped microcapsules nearby other bigger agglomerates of unequal shapes

Oxidative stability: The difference between antiradical and antioxidant is not always clear because the antioxidant activity points to the capability to suppress the oxidation process which is often includes a set of diverse reactions[39]. The time taken until a sudden increase in conductivity is called oil stability index (OSI) or induction period (IP). Pure olive oil (OO) was used as reference oil to compare the OSI of HO and Ramly seed oil (RO). Although virgin olive oil has being acknowledged to be of utmost importance among different vegetable oils because of its high content of phenolic compounds, its OSI measured by Rancimat was the lowest (14.41%), whereas, HO showed the highest OSI value (40.44%) (Fig. 5). The results of the present study were in consistence with a previous study[30].

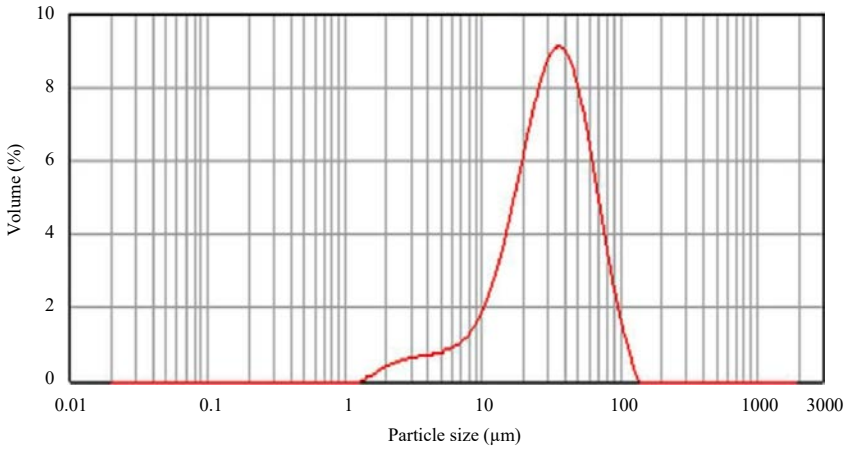

Fig. 4: Particles morphology and size scattering of date seed oil powder

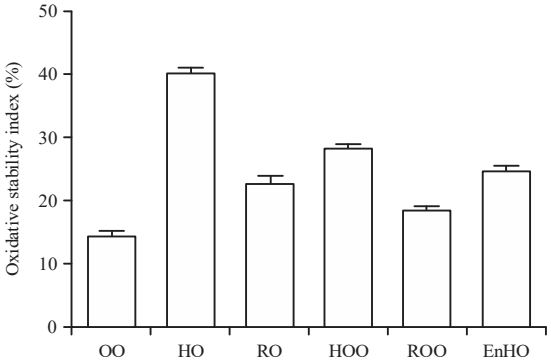

Fig. 5: Oxidative stability of different seed oils and olive-seed oil mixtures

Data are Mean±SD, n = 3. OO: Olive oil, HO: Helwa oil, RO: Ramly oil, HOO: Helwa/olive oil mixture, ROO: Ramly/olive oil mixture, EnHO: Encapsulated Helwa/olive oil mixture

CONCLUSION

Date seed oil could represent an important value-added by-product to help filling the gap between production and consumption of edible oils. Due to their high content of polyphenols and tocols, these oils can stabilize other oils and protect against oxidation. The addition of encapsulated date seeds oil to olive oil provided additional protection and improved the nutritional value and functionality of the final oil product. The findings of this study showed the high relative percentages of oleic acid in date seed oils. In addition, date seed oils are more yellow-colored than other vegetable oils and can confer protection against UV light and prevent cellular damage. These interesting characteristics of date seed oil make it a promising candidate for use in cosmetics and food industries.

SIGNIFICANCE STATEMENT

This study confers new information that the addition of micro encapsulated date seed oil to olive oil can be beneficial for improving the nutritional value and functionality of the final olive oil product and protect it against oxidation. In addition, this study shows the interesting characteristics of date seed oil, making it a promising candidate for the use in cosmetics and food industries.

ACKNOWLEDGMENT

The authors would like to thank the Olive Research Center, Deanship of Postgraduate studies and Research, Jouf University (Saudi Arabia) for the funding this study (Grant No. 1-4-37).

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