http://www.pjbs.org



ISSN 1028-8880

Pakistan Journal of Biological Sciences



Pakistan Journal of Biological Sciences

ISSN 1028-8880 DOI: 10.3923/pjbs.2019.318.326



Research Article Preparation and Properties Nano-encapsulated Wheat Germ Oil and its Use in the Manufacture of Functional Labneh Cheese

¹Tarek Nour Soliman, ¹Atif Farrag Farrag, ²Hamdy Abdel-Hady Zahran and ¹Mohamed EL-Hossieny Abd El-Salam

¹Department of Dairy, National Research Centre, 12622 Dokki, Cairo, Egypt ²Department of Fats and Oils, National Research Centre, 12622 Dokki, Cairo, Egypt

Abstract

Background and Objective: There is a growing interest to develop novel versions of traditional dairy products by upgrading their health properties. The use of wheat germ oil (WGO) known by its health-promoting effects, in the fortification of dairy products such as Labneh represents a challenge. This study was aimed to prepare nano-encapsulated WGO and to develop Labneh enriched with nano-encapsulated WGO. **Materials and Methods:** The WGO was encapsulated in casein micelles by pH changes and ultra-sonication. Transmission electron microscopy and laser light scattering characterized the size and shape of the prepared WGO capsules and their zeta potential was determined. The antioxidant activity and oxidative stability of encapsulated WGO, respectively. Labneh was made from standardized (3% fat) milk and by replacement 50% of milk fat with free and encapsulated WGO, respectively. Labneh was analyzed for gross composition, textural parameters, colour and sensory properties during cold storage for 20 days. **Results:** High encapsulation efficiency (>95%) of different levels of WGO (0.3 to 1.2%) in casein micelles was obtained. The encapsulated WGO had a spherical shape and nano sizes. The particle sizes increased with the increase of the encapsulated level of WGO. The encapsulated WGO retained high DPPH scavenging activity and exhibited high oxidative stability. Labneh made with encapsulated WGO had composition and quality comparable to the control. **Conclusion:** Functional Labneh of acceptable quality and high antioxidant activity could be prepared by replacement of 50% of milk fat with encapsulated WGO.

Key words: Labneh, casein micelles, encapsulation, oxidative stability, wheat germ oil

Citation: Tarek Nour Soliman, Atif Farrag Farrag, Hamdy Abdel-Hady Zahran and Mohamed EL-Hossieny Abd El-Salam, 2019. Preparation and properties nano-encapsulated wheat germ oil and its use in the manufacture of functional labneh cheese. Pak. J. Biol. Sci., 22: 318-326.

Corresponding Author: Tarek Nour Soliman, Department of Dairy, National Research Centre, 12622 Dokki, Cairo, Egypt Tel: 0020 1155945511 Fax: 0020 233370931

Copyright: © 2019 Tarek Nour Soliman *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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

The growing interest in functional foods created the need to upgrade the health properties of traditional foods particularly dairy products. This can be done by the inclusion of health-promoting ingredients in their composition. Labneh (concentrated yoghurt) is a product of the soft and smooth body, cream/white colour, good spreadability and slightly acidic, clean flavour and better nutritional value than yoghurt¹. Attempts have been made to improve the health properties of Labneh by the inclusion of probiotic and conjugated linoleic acid² and moringa leaves³, but no study has been cited on upgrading the antioxidant properties of labneh.

The nutritional and health properties of polyunsaturated marine and vegetable oils including wheat germ oil encouraged their utilization in the development of functional foods. However, their susceptibility to oxidative deterioration and formation of degradation products of unpleasant taste and flavour limits their full potential utilization in foods⁴. Encapsulation can offer a feasible way to overcome these problems and to develop functional foods rich in polyunsaturated oils⁵.

Wheat germ oil (WGO) is a polyunsaturated oil extracted from wheat germ, a by-product of flour milling⁶. It is high in α -tocopherol known by its antioxidant and vitamin E activities⁷ and policosanols especially octacosanol⁸, which were reported to improve the physical performance of consumers⁹. In addition, WGO was reported to be useful in lowering blood cholesterol levels, management of chronic inflammatory reactions and neurological disorders¹⁰. Therefore, WGO has been used in the treatment of diseases involving oxidative damage^{11,12}. However, WGO is prone to rapid oxidation due to its high polyunsaturated fatty acid (PUFA) content. Encapsulation has been reported to improve the oxidative stability of WGO¹³⁻¹⁶ and its use in the fortification of foods.

Casein and caseinate have been used in nano-encapsulation hydrophobic compounds for their unique structure and functional properties. Caseins have open structure lacking any rigid secondary structure¹⁷. In addition, they have a significant number of hydrophobic amino acid residues, which allow their strong adsorption at the droplet surface^{18,19}. Furthermore, they can form a thicker interfacial layer around the fat droplets compared with whey proteins, which enhance the stability of the formed emulsion^{20,21}. These characteristics are the main reasons for their high encapsulation efficiency²² and the oxidative stability of entrapped materials²³. Also, the open structure of caseins results in easier access of gastric proteases for their enhanced digestibility and the release of the encapsulated substances²⁴. Reassembled casein micelles have been used successfully in nano-encapsulation of hydrophobic molecules such as²⁵ vitamin D₂ and ω -3 polyunsaturated fatty acids²⁶.

Alkali treatment was reported to disrupt the natural casein micelles in a way depending on the pH and that the micellar structure can be restored by adjusting pH to the normal pH of milk^{27,28}. At higher pH, the size of casein micelle becomes larger and swelled due to electrostatic repulsion between protein monomers²⁹. In addition, it has been reported that sonication at high pH values (6.6 to 12) can significantly affect the micellar size via breaking non-peptide bonds of the re-assembled casein³⁰. Based on these characteristics a simple method has been recently developed for nano-encapsulation of hydrophobic materials in natural casein micelles (CM) without the need for its separation from milk³¹.

Encapsulation was necessary to protect the addition of wheat germ oil from oxidation and to prevent any deleterious effect of the added oil on the organoleptic properties of the product. Therefore, the objective of this work was to study the effect of addition encapsulated wheat germ oil via milk proteins on the manufacture and characteristics of functional Labneh cheese.

MATERIALS AND METHODS

Materials: Imported low heat skim milk powder (SMP) DAIRYAMERICA, Inc. California, USA (34% protein, 51% lactose, 0.8% fat, 8.2% ash and 4% moisture). Wheat germ oil (WGO) was obtained from the unit of oil extraction, National Research Centre (NRC), Cairo, Egypt. Lactic acid strains: Streptococcus thermophilus CH-1 was obtained from Chr. Hansen's Lab., Denmark and Lactobacillus delbrueckii subsp. bulgaricus Lb-12 was provided by Northern Regional Research Laboratory, Illinois, USA. Cultures were propagated in sterilized reconstituted skim milk (10%). A mixture (1:1) of the two strains was used as a starter in the manufacture of labneh. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma (St. Louis, MO, USA). Chemicals such as; concentrated HCl, sodium hydroxide, petroleum ether (B.P. 40-60°C), diethyl ether, Folin-ciocalteu reagent, gallic acid and ammonia (25%) were purchased from Merck Chemicals Co. (Darmstadt, Germany). All employed chemicals exhibited Analar or equivalent quality. This study was done between November, 2017 and August, 2018.

Methods

Nano-encapsulation of WGO: The method described by Ghasemi and Abbasi³¹ for encapsulation of oils in CM by pH manipulation and ultra-sonication was followed.

Total phenolic content (TPC): Total phenol content of WGO samples were determined by spectrophotometer at 625 nm using the Folin-ciocalteu reagent according to the method described by Mohamed *et al.*³². The solution of WGO extract (0.5 mL), 20 mL of distilled water and 0.625 mL of the Folin-ciocalteu reagent was added to a 25 mL volumetrically flask. After 3 min, 2.5 mL of saturated solution of Na₂CO₃ (7%) were added. The contents were mixed vigorously and diluted to adjusted volume with distilled water. After 1 h, the absorbance of the sample was measured at 625 nm against a blank using a double-beam ultraviolet-visible spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan). Gallic acid served as a standard for preparing the calibration curve and ranged from 2.5 to 20 μ g/25 μ L of the assay solution.

Antioxidant activity: Antioxidant activity of the WGO samples was determined by the utilization of DPPH radical scavenging activity³³. Where WGO and encapsulated samples extracts (0.1 mL) were diluted and mixed with 3.9 mL methanolic solution of DPPH (0.1 mM), after incubation for 30 min in dark at room temperature, the absorbance at 517 nm was measured. A sample of free DPPH solution was used as a control. The following equation was used to determine the DPPH radical scavenging activity (%):

DPPH scavenging activity (%) =
$$\frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Characteristic microstructure: Samples were prepared for transmission electron microscopy (TEM) by fixation with glutaraldehyde and phosphotungstic acid solution (2% at pH 7.2) as described by Moslehishad and Ezzatpanah³⁴. The samples were examined by TEM using a JEOL JEM-1400 Plus TEM with an accelerating voltage of 100 kV at a magnification of 200,000x.

Encapsulation efficiency (EE): The total encapsulated and free oil contents were measured by Rose Gottlieb method³⁵. Free oil was determined by the same method after removal of the encapsulated oil by isoelectric precipitation and encapsulated oil was calculated by difference from total oil. The EE was calculated as the percentage of encapsulated/total oil²⁰.

Size distribution: The size distribution of samples were carried out at 25 ± 0.1 EC using nano ZS/ZEN3600 Zetasizer

(Malvern Instruments Ltd., UK) with a He/Ne laser (λ = 633 nm), scattering angle 90° scattering optics and refractive index of 1.35. Samples were diluted and filtered through 0.45 µm membrane (Millipore, USA) to obtain a count rate in the appropriate range 100-450 nm to avoid multiple scattering phenomena due to inter-particle interaction. Immediately, the diluted sample was transferred into the polystyrene cuvette for size determination and polydispersity index (PDI) were recorded by dynamic light scattering (DLS) as described by Giroux *et al.*³⁶.

Oxidative stability of nano-encapsulated WGO: The oxidative stability of encapsulated and free WGO was determined by heat treatment and UV light exposure as described by Ghasemi and Abbasi³¹.

Peroxide value: Peroxide value (PV) of WGO free and encapsulated samples were determined according to methods of AOAC³⁷ and Shantha and Decker³⁸, respectively.

Manufacture of Labneh: The fat content of reconstituted skim milk (10% w/w) was adjusted to 3% using fresh cream (control) and by replacement 50% of milk fat with free WGO (treatment 1, T1) and nano-encapsulated WGO (treatment 2, T2). The prepared milk from the different treatments was homogenized, heated (85°C/5 min) and cooled to 42°C, inoculated with 3% of the starter culture and then left until complete coagulation. The coagulum was kept cool in the refrigerator overnight, mixed thoroughly with 1% (w/w) of sodium chloride transferred to a cheese cloth bags and then hung in the refrigerator for 12 h to allow whey drainage. The fresh Labneh was scoped into 100 g plastic containers and kept at 5°C for 20 days.

Chemical analysis of Labneh: The total solids (by dry oven at $105 \degree C/3 h$), fat (by Gerber method), protein (Kjeldahl method) and ash (by muffle furnace at $550 \degree C/5 h$) contents of labneh were determined as described by earlier methods^{38,39} and lactose content was calculated by difference. The pH value was measured using a digital pH-meter with a combined electrode (HANNA).

Textural analysis of Labneh: The textural properties of Labneh from different treatments were assessed using the textural analyzer (Mult- test 1dMemesin, Food Technology Corporation, Slinfold, W. Sussex, UK.) equipped with a 25 mm diameter perplex conical shaped probe. The texture profile analysis (TPA) was done on Labneh samples as described by Eman *et al.*⁴⁰. The textural parameters were calculated from the force-time curve according to the definition given by IDF⁴¹.

Measurements of colour parameters: The colour parameters (L, a and b) of Labneh were measured using Hunter colorimeter Model D2s A-2 (Hunter Assoc. Lab. Inc. Va, USA) following the instruction of the manufacturer⁴².

Sensory evaluation: The organoleptic properties of Labneh were assessed by 20 panelists of the experienced staff members of Dairy Department, NRC as described by El-Shafei *et al.*⁴². Samples were evaluated for flavour (out of 50 points), body and texture (out of 40 points) and appearance (out of 10 points).

Statistical analyses: The analyses of prepared samples were conducted at least in triplicates, comparisons of the treatments were completed by one-way ANOVA and Tukey's tests by SPSS, ver. 16.0 statistics programs. A 95% minimum confidence level was taken for all statistical analyses⁴³.

RESULTS

Encapsulation efficiency of nano-encapsulated WGO: Encapsulation efficiency (EE) was shown in Table 1, it was ranged between 96.31-99.44% for all loaded WGO percentages in CM and significant differences (p<0.05) were found between treatments with the lowest EE for 1.2% WGO.

Characteristics of nano-encapsulated WGO: The sizes of CM and the encapsulated WGO followed a normal distribution curve (Fig. 1), but with broader right side in case of encapsulated WGO indicating the presence of large size particles. This was also apparent from comparing the mean particle size and the PDI of the CM and the encapsulated WGO (Table 2). The particle size distribution was in nano size for all samples, which ranged between 244 and 335 nm. Transmission electron micrograph (TEM) showed that the encapsulated WGO retained a core/shell structure (Fig. 2), whereas, the WGO occupied the core with an average diameter of 171 nm while casein formed a 55 thick shell. The average size of WGO loaded particles was 285 nm being less than that determined by laser light scattering being $(\lambda = 633 \text{ nm})$. This can be attributed to dehydration step of TEM leading to contraction of the examined particle.

Stability of nano-encapsulated WGO: Table 3 showed that the used free WGO had TPC content of 16.50 mg g⁻¹ and 5.44% DPPH scavenging activity. Increasing the percentage of WGO encapsulated to 1.2% led to increasing significantly (p<0.05) of TPC and DPPH scavenging activity reached of 17.35 mg g⁻¹ and 6.18%, respectively. This means that the encapsulation of WGO (till 1.2%) keep and improvement of

TPC and DPPH scavenging activity for it and it can be utilized for supplementation (or fortification) and production of functional dairy products like Labneh.

Table 1: Encapsulation efficiency of WGO and percentage of extracted oil at different pH

WGO emulsified	Extracted oil (%)		
in RSM (%)	pH 6.7	pH 8	Encapsulation efficiency
0.3	0.30±0.01ª	0.30±0.02ª	98.79±0.08 ^b
0.6	$0.61 \pm 0.02^{\text{b}}$	$0.60 \pm 0.03^{\text{b}}$	99.27±0.02 ^c
0.9	0.90±0.03°	0.88±0.04°	99.44±0.02°
1.2	1.20 ± 0.03^{d}	1.19±0.03 ^d	96.31±0.21ª

Means with different superscript letters in the same column are significantly different (p<0.05)

Table 2: Particle size (nm) of casein micelles and polydispersity index (PDI) of treated samples

treated sumples		
Samples	Size (nm)	Calculated PDI
Reconstituted milk	279±68 ^b	0.155
Ultra-sonicated milk	244±78ª	0.165
0.30% WGO	295±59 ^b	0.220
0.60% WGO	329±32°	0.149
0.90% WGO	323±54°	0.240
1.20% WGO	335±83°	0.233

Means with different superscript letters in the same column are significantly different (p<0.05)



Fig. 1: Size distribution curves of casein micelles and encapsulated WGO



Fig 2: Transmission electron micrograph of encapsulated WGO particle



Fig. 3: Changes in the DPPH scavenging activity of free WGO and encapsulated after exposure to UV up to 6 h



Fig. 4: Changes in the peroxide value of free WGO and encapsulated after exposure to UV up to 18 h

Table 3: Total phenolic compounds (TPC) and DPPH scavenging activity (%) of free WGO and encapsulated WGO

WGO (%)	TPC (mg g^{-1})	Antioxidant activity (%)
Free WGO	16.50±1.41	5.44±0.08
0.3%	6.62±0.83ª	2.33±0.32ª
0.6%	11.28±0.15 ^b	4.27±0.15 ^b
0.9%	16.48±1.02°	6.14±0.25°
1.2%	17.35±0.67°	6.18±0.11°

Means with different superscript letters in the same column are significantly different (p<0.05)

Table 4: Chemical composition of fresh Labneh (control) and fortified samples

Exposure to UV decreased the DPPH scavenging activity of both free and encapsulated WGO (Fig. 3). However, the decrease was more pronounced in free WGO. In addition, Fig. 4 showed an accelerated increase in the peroxide value of free WGO during the exposure to UV radiation while the encapsulated WGO maintained good oxidative stability up to 18 h of UV exposure.

Preparation and properties of WGO fortified Labneh: The addition of encapsulated and nano-encapsulated WGO in the manufacture of Labneh does not affect the coagulation time of milk indicating that it had no adverse effect on the activity of the used starter. Slight differences were found in the fat, protein and ash contents of Labneh from the different treatments (Table 4) ranged from 8.73 and 9.03, 9.93 and 10.10, 1.51 and 1.57%, respectively. This can be attributed to the slight differences in their total solid contents was ranged from 29.49 and 30.55%.

Physical properties of WGO fortified Labneh organoleptic

of WGO fortified labneh: The inclusion of encapsulated WGO had an insignificant effect (p<0.05) on the colour parameters L* was 88.43 of Labneh as compared to the control 88.30 (Table 5). However, the use of non-encapsulated WGO decreased the whiteness (L-value) 84.04 and increased the redness (a-value) to 1.26 and yellowness (b-value) 25.65 of Labneh as compared to the control was 88.30, 0.60 and 18.30, respectively.

During the storage of all samples Labneh, acidity was continued to develop which decreased slightly the pH of Labneh (Table 6).

Tuble II chemi	usie i. chemical composition of nesh Edonen (control) and fortance samples								
Samples	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	Lactose (%)				
A	30.03±0.38 ^{ab}	8.90±0.10 ^{ab}	10.01±0.02 ^b	1.55±0.05ª	4.08±0.03 ^{ab}				
В	29.49±0.29ª	8.73±0.15ª	9.93±0.05ª	1.51±0.02ª	4.15±0.05 ^b				
С	30.55±0.42 ^b	9.03±0.12 ^b	10.10±0.05°	1.57±0.03ª	4.03±0.02ª				

Means with different superscript letters in the same column are significantly different (p<0.05), A: Labneh (control), B: Labneh containing non-encapsulated WGO, C: Labneh containing encapsulated WGO

Table 5: Colour parameters of fresh Labneh (control) and fortified samples

	· , , , , , , , , , , , , , , , , , , ,		
Samples	L*	a*	b*
A	88.30±0.02 ^b	0.60 ± 0.04^{b}	18.3±0.08ª
В	84.04±0.14ª	1.26±0.01°	25.65±0.02°
C	88.43±0.02 ^b	0.24±0.02ª	21.02±0.02 ^b

Means with different superscript letters in the same column are significantly different (p<0.05), *L: Value represents darkness from black (0) to white (100), *a-value represents colour ranging from red (+) to green (-) and *b-value represents yellow (+) to blue (-), A: labneh (control), B: Labneh containing non-encapsulated WGO, C: Labneh containing encapsulated WGO

Pak. J. Biol. Sci., 22 (7): 318-326, 2019

Table 6: Changes in the pH and a	cidity (%) of fresh labneh («	control) and fortified same	ples during storage
2 1		<i>i</i>	5 5

	Fresh	Fresh		7 days		14 days		21 days	
Samples	рН	Acidity	рН	Acidity	рН	Acidity	рН	Acidity	
A	4.82 ^b	0.55ª	4.77ª	0.63ª	4.59ª	0.79ª	4.42ª	0.90ª	
В	4.73ª	0.67 ^b	4.64 ^b	0.77ª	4.58ª	0.81ª	4.38ª	0.99ª	
С	4.84 ^b	0.53ª	4.77ª	0.59ª	4.61ª	0.78ª	4.42ª	0.90ª	

Means with different superscript letters in the same column are significantly different (p<0.05), A: Labneh (control); B: Labneh containing non-encapsulated WGO, C: Labneh containing encapsulated WGO

Table 7: Texture profile analysis of fresh labneh (control) and fortified samples during s	storage
--	---------

Treatments	Storage	Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N×mm)
A	Fresh	12.36	0.998	0.575	7.11	7.09
	21 days	11.76	0.976	0.585	6.88	6.71
В	Fresh	11.21	0.932	0.491	5.50	5.13
	21 days	10.85	0.925	0.524	8.54	5.90
С	Fresh	13.25	1.050	0.506	6.70	7.04
	21 days	12.50	0.988	0.537	6.71	6.63

N: Newton, A: Labneh (control), B: Labneh containing non-encapsulated WGO, C: Labneh containing encapsulated WGO

Table 8: Sensory scores of fresh labneh (control) and fortified samples during storage

	Flavour (50)		Body and text	Body and texture (40)		Appearance (10)		Total (100)	
Samples	 Fresh	Stored	Fresh	Stored	 Fresh	Stored	Fresh	Stored	
A	45.8±1.0 ^b	41.5±1.3ª	35.5±1.0ª	32.80±1.0 ^{ab}	8.8±1.3 ^b	8.30±1.0 ^{ab}	90.0±2.9 ^b	82.5±1.3 [♭]	
В	42.8±1.0ª	42.0 ± 0.8^{ab}	35.8±1.0ª	31.00±1.4ª	7.0±0.8ª	7.00±0.82ª	85.5±2.9ª	80.0±1.4ª	
С	46.8±1.0 ^b	43.5±0.6 ^b	37.8±1.3 ^b	33.8 ± 1.0^{b}	8.8±0.5 ^b	$8.75 \pm 0.50^{\text{b}}$	93.3±1.5 ^b	86.0±1.2°	
	1 1/00	the second second second							

Means with different superscript letters in the same column are significantly different (p<0.05), A: Labneh (control), B: Labneh containing non-encapsulated WGO, C: Labneh containing encapsulated WGO

Organoleptic of WGO fortified labneh: After cold storage (21 days) the hardness and springiness of Labneh from different treatments decreased slightly (Table 7). Sensory evaluation (Table 8) revealed that Labneh made with the encapsulated WGO ranked higher scores for flavour, body and texture, appearance and total scores than the control while that made with nano-encapsulated WGO gets lower scores for all sensory attributes. Labneh from all treatments gets lower scores at the end of the storage period (21 days).

DISCUSSION

In the present study, the difference of total phenolic compound content may still contribute the difference of scavenging DPPH capability, which, concentrations 0.3<0.6<0.9<1.2% in TPC and antioxidant activity (Table 3). The antioxidant activity of WGO could be attributed to its content of tocopherols⁴⁴. Several studies suggested that the total phenolics content might have a positive correlation with antioxidant activity^{43,45-46}.

Selection of the wall material is an important step in the encapsulation of bioactive ingredients and oils⁶. In the present study, encapsulation of WGO was carried out in natural casein micelles³¹. In addition to the favorable properties of casein micelles as wall material for encapsulation of WGO, it has the advantage that encapsulation can be done without the need to separate casein micelles from milk and the obtained

capsules had nano sizes being less than the previous reports¹ has resulted in Salem et al.³ Chan et al.¹⁴ and Karadeniz et al.¹⁶. However, smaller WGO emulsion size encapsulated in maltodextrin/Arabic gum/WPC as wall material being 10 nm was reported¹⁵, which is practically difficult to achieve. The WGO was almost completely recovered in the acid precipitated casein due to the increased hydrophobic forces of casein at its isoelectric point which resulted in the high encapsulation efficiency (EE) obtained. The obtained EE are in agreement with those reported by Ghasemi and Abbasi³¹, but higher than those found in the encapsulation of WGO in maltodextrin/whey protein concentrate system¹² or in sodium alginate¹⁴, maltodextrin/WPC/Arabic gum¹⁵ and in different wall materials¹⁶. This can be attributed to the high hydrophobic affinity of casein micelles¹⁷. Ultrasonic treatment of reconstituted skim milk (RSM) reduced significantly (p<0.05) the average size of casein micelles. This may be attributed to possible disaggregation of agglomerated particles formed during the spray drying of milk. On the other hand, entrapment of WGO skim increased the micellar size and this increase was proportional to the WGO level, which indicated clearly the entrapment of WGO within the casein micelle structure. This was confirmed also by TEM examination. The present results confirmed those reported by Ghasem and Abbasi³¹, who found that oil entrapment increased the micellar size from 306 to 437 nm.

Replacement of 50% of milk fat in Labneh with free and encapsulated WGO had a slight effect of the gross composition of the obtained product. This can be understood from the same percentage of fat in all products irrespective of the nature of fat/oil used. However, differences were found in the colour of Labneh from different treatments. Labneh made with free WGO was characterized by darker colour (low L parameter) and high colour parameters (a and b) which can be attributed to the yellow pigments (Flavonoids, xanthophyll and xanthophyll esters) present in WGO47,48. Encapsulation masked the colour of WGO, which was responsible for the comparable colour of Labneh containing WGO and the control. In addition, the use of free WGO in replacement of milk fat decreased slightly the hardness of Labneh. This can be explained by the decrease in the hardness of labneh fat on the addition of the free WGO. Encapsulated WGO increased slightly the hardness due to its nano size and entrapment in casein micelles. During storage, the hardness of labneh decreased slightly which can be attributed to proteolysis in its protein matrix³. The inclusion of free WGO in labneh imparted slight oily taste in the product, which explains the low flavour scores in this treatment, while encapsulation masked the oily taste of WGO. In the meantime, the encapsulated WGO can be used to fortify traditional foods as a mean to develop functional products of improved antioxidant activity of health properties.

CONCLUSION

This study discovered the wheat germ oil (WGO) can be encapsulated in natural casein micelles without the need to separate micelles from milk. The encapsulated WGO particles had spherical shapes, Nano sizes and characterized by high antioxidant activity and good oxidative stability. That can be beneficial for produced functional labneh of high antioxidant activity and similar quality to the traditional labneh can be prepared by replacement 50% of milk fat with encapsulated WGO. On the other hand, the physicochemical characteristics, as well as, the organoleptic properties of produced labneh were enhanced by the fortification with encapsulated WGO. This study will help the researchers to uncover the critical areas of functional dairy products that many researchers were not able to explore. Thus this the first study to report the use of encapsulated WGO in a food commodity.

SIGNIFICANCE STATEMENT

Wheat germ oil (WGO) can be encapsulated in natural casein micelles without the need to separate micelles from

milk. The encapsulated WGO particles had spherical shapes, nano sizes and characterized by high antioxidant activity and good oxidative stability. Functional labneh of high antioxidant activity and similar quality to the traditional labneh was prepared by replacement 50% of milk fat with encapsulated WGO.

ACKNOWLEDGMENT

A lot of thanks to all staff members of the internal project No. 11040104 funded by National Research Centre, Dokki, Cairo Egypt which support and funded this work.

REFERENCES

- Nsabimana, C., B. Jiang and R. Kossah, 2005. Manufacturing, properties and shelf life of labneh: A review. Int. J. Dairy Technol., 58: 129-137.
- Abd El-Salam, M.H., A.R. Hippen, K. El-Shafie, F.M. Assem and H. Abbas *et al.*, 2011. Preparation and properties of probiotic concentrated yoghurt (labneh) fortified with conjugated linoleic acid. Int. J. Food Sci. Technol., 46: 2103-2110.
- Salem, A.S., W.M. Salama, A.M. Hassanein and H.M. El Ghandour, 2013. Enhancement of nutritional and biological values of Labneh by adding dry leaves of *Moringa oleifera* as innovative dairy products. World Applied Sci. J., 22: 1594-1602.
- Bakry, A.M., S. Abbas, B. Ali, H. Majeed, M.Y. Abouelwafa, A. Mousa and L. Liang, 2016. Microencapsulation of oils: A comprehensive review of benefits, techniques and applications. Compr. Rev. Food Sci. Technol., 15: 143-182.
- Singh, H., 2016. Nanotechnology applications in functional foods; opportunities and challenges. Prev. Nutr. Food Sci., 21: 1-8.
- Megahad, O.A. and O.S. El Kinawy, 2002. Studies on the extraction of wheat germ oil by commercial hexane. Grasasy Y Aceites, 53: 414-418.
- Dunford, N.T., 2012. Food and Industrial Bioproducts and Bioprocessing. John Wiley and Sons, USA, ISBN: 9780813821054, Pages: 374.
- Irmak, S. and N.T. Dunford, 2005. Policosanol contents and compositions of wheat varieties. J. Agric. Food Chem., 531: 5583-5586.
- Kim, H., S. Park, D.S. Han and T. Park, 2003. Octacosanol supplementation increases running endurance time and improves biochemical parameters after exhaustion in trained rats. J. Med. Food, 6: 345-351.
- Reddy, B.S., Y. Hirose, L.A. Cohen, B. Simi, I. Cooma and C.V. Rao, 2000. Preventive potential of wheat bran fractions against experimental colon carcinogenesis: Implications for human colon cancer prevention. Cancer Res., 60: 4792-4797.

- 11. Anwar, M.M. and N.E. Mohamed, 2015. Amelioration of liver and kidney functions disorders induced by sodium nitrate in rats using wheat germ oil. J. Radiat. Res. Applied Sci., 8: 77-83.
- 12. Aniolowska, M., H. Zahran and A. Kita, 2016. The effect of pan frying on thermooxidative stability of refined rapeseed oil and professional blend. J. Food Sci. Technol., 53: 712-720.
- 13. Yazicioglu, B., S. Sahin and G. Sumnu, 2015. Microencapsulation of wheat germ oil. J. Food Sci. Technol., 52: 3590-3597.
- 14. Chan, L.W., L.T. Lim and P.W.S. Heng, 2000. Microencapsulation of oils using sodium alginate. J. Microencapsul., 17: 757-766.
- 15. Salem, R.H. and A.M. Khalifa, 2018. Preparation and characteristics of micro and nano wheat germ oil capsules. World J. Dairy Food Sci., 13: 37-45.
- 16. Karadeniz, M., S. Sahin and G. Sumnu, 2018. Enhancement of storage stability of wheat germ oil by encapsulation. Ind. Crops Prod., 114: 14-18.
- 17. Holt, C. and L. Sawyer, 1988. Primary and predicted secondary structures of the caseins in relation to their biological functions. Protein Eng., 2: 251-259.
- 18. Swaisgood, H.E., 2013. Chemistry of the Caseins. In: Advanced Dairy Chemistry-Proteins. McSweeney, P.L.H. and P.F. Fox (Eds.), Vol. 1A, Springer, Boston, MA, ISBN: 978-1-4419-8602-3, pp: 139-201.
- Graham, D.E. and M.C. Phillips, 1979. Proteins at liquid interfaces: II. Adsorption isotherms. J. Colloid Interface Sci., 70: 415-426.
- 20. Dalgleish, D.G., 1993. The sizes and conformations of the proteins in adsorbed layers of individual caseins on latices and in oil-in-water emulsions. Colloids Surf. B: Biointerfaces, 1: 1-8.
- 21. Fang, Y. and D.G. Dalgleish, 1993. Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. J. Colloid Interface Sci., 156: 329-334.
- Hogan, S.A., B.F., McNamee, E.D. O'Riordan and M. O'Sullivan, 2001. Microencapsulating properties of sodium caseinate. J. Agric. Food Chem., 49: 1934-1938.
- 23. Hu, M., D.J. McClements and E.A., Decker, 2003. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate and soy protein isolate. J. Agric. Food Chem., 51: 1696-1700.
- Fox, P.F., 2003. Milk Proteins: General and Historical Aspects.
 In: Advanced Dairy Chemistry-1Proteins. Fox, P.F., P.L.H. McSweeney (Eds.), Springer, Boston, MA, ISBN: 978-1-4419-8602-3 pp: 1-48.
- 25. Semo, E., E. Kesselman, D. Danino and Y.D. Livney, 2007. Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocolloids, 21: 936-942.
- Zimet, P., D. Rosenberg and Y.D. Livney, 2011. Re-assembled casein micelles and casein nanoparticles as nano-vehicles for ω-3 polyunsaturated fatty acids. Food Hydrocolloids, 25: 1270-1276.

- Huppertz, T., B. Vaia and M.A. Smiddy, 2008. Reformation of casein particles from alkaline-disrupted casein micelles. J. Dairy Res., 75: 44-47.
- Sinaga, H., N. Bansal and B. Bhandari, 2017. Effects of milk pH alteration on casein micelle size and gelation properties of milk. Int. J. Food Propert., 20: 179-197.
- 29. Liu, Y. and R. Guo, 2008. pH-dependent structures and properties of casein micelles. Biophys. Chem., 136: 67-73.
- Madadlou, A., M.E. Mousavi, Z. Emam-Djomeh, M. Ehsani and D. Sheehan, 2009. Sonodisruption of re-assembled casein micelles at different pH values. Ultrason. Sonochem., 16: 644-648.
- 31. Ghasemi, S. and S. Abbasi, 2014. Formation of natural casein micelle nanocapsule by means of pH changes and ultrasound. Food Hydrocolloids, 42: 42-47.
- 32. Mohamed, F.A.E.F., H.H. Salama, S.M. El-Sayed, H.S. El-Sayed and H.A.H. Zahran, 2018. Utilization of natural antimicrobial and antioxidant of *Moringa oleifera* leaves extract in manufacture of cream cheese. J. Biol. Sci., 18: 92-106.
- 33. Zahran, H.A. and H.M. Soliman, 2018. UPLC-Q-TOF/MS screening of bio-active compounds extracted from olive mill solid wastes and their effect on oxidative stability of purslane seed oil. Curr. Sci. Int., 7: 307-319.
- 34. Moslehishad, M. and H. Ezzatpanah, 2010. Transmission electron microscopy study of casein micelle in raw milk with different somatic cell count levels. Int. J. Food Prop., 13: 546-552.
- 35. ISO., 2010. Milk-determination of fat content-gravimetric method (Reference method). ISO 1211: 2010, IDF 1: 2010, International Organization of Standardization (ISO), Geneva.
- 36. Giroux, H.J., J. Houde and M. Britten, 2010. Preparation of nanoparticles from denatured whey protein by pH-cycling treatment. Food Hydrocolloids, 24: 341-346.
- AOAC., 2000. Official Methods of Analysis of AOAC International. 17th Edn., AOAC International, Gaitherburg, MD., USA., ISBN-13: 9780935584677, Pages: 2200.
- Shantha, N.C. and E.A. Decker, 1994. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J. AOAC Int., 77: 421-424.
- 39. Ardo, Y. and A. Polychroniadou, 1999. Laboratory Manual for Chemical Analysis of Cheese: Improvement of the Quality of the Production of Raw Milk Cheeses. Publications Office, Luxembourg, ISBN: 9282865991, Pages: 122.
- Eman, E.H., M.S. Sherif, A.A. Sameh and M.Z. Hamid, 2017. Physicochemical and sensory properties of Labneh fortified with *Salvia officinalis*. Alexandria J. Food Sci. Technol., 38: 761-769.
- 41. IDF., 1991. Rheological and fracture properties of cheeses. Bulletin of the IDF No. 268, International Dairy Federation, Brussels, Belgium. http://www.fil-idf.org/Public/Publication. php?ID=28509&ajax=true

- 42. El-Shafei, K., F.M. Assem, A.B. Abd-El-Khalek and O.M. Sharaf, 2011. Production of high quality functional labneh cheese using exopolysaccharides producing encapsulated *Lactobacillus bulgaricus*. J. Life Sci., 5: 120-128.
- 43. SPSS., 2007. SPSS Professional Statistics (Version 16). SPSS Inc., Chicago IL, USA.
- 44. Emmons, C.L., D.M. Peterson and G.L., Paul, 1999. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. *In vitro* antioxidant activity and contents of phenolic and tocol antioxidants. J. Agric. Food Chem., 47: 4894-4898.
- 45. Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 46: 4113-4117.
- Lee, Y.Y., H.M., Park, T.Y. Hwang, S.L. Kim and M.J. Kim *et al.*, 2015. A correlation between tocopherol content and antioxidant activity in seeds and germinating seeds of soybean cultivars. J. Sci. Food Agric., 95: 819-827.
- 47. Mahmoud, A.A., A.A.A. Mohdaly and N.A.A. Elneairy, 2015. Wheat germ: An overview on nutritional value, antioxidant potential and antibacterial characteristics. Food Nutr. Sci., 6: 265-277.
- Ghafoor, K., M.M. Ozcan, F. Al Juhaimi, E.E. Babiker, Z.I. Sarker, I.A.M. Ahmed and M.A. Ahmed, 2017. Nutritional composition, extraction and utilization of wheat germ oil: A review. Eur. J. Lipid Sci. Technol., Vol. 119. 10.1002/ejlt.201600160.