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Research Article
Production of Functional Processed Cheese Supplemented with Nanoliposomes of Mandarin Peel Extract

Tamer Mohammed El-Messery, Marwa Mohamed El-Said and Eman Sayed Abdelkader Farahat

Department of Dairy Sciences, National Research Centre, Giza, Egypt

Abstract

Background and Objective: The Mandarin fruit is a wonderful source of essential dietary nutrients. The liposome is an encapsulation method to incorporate the phenolics in functional food. The objective of this study was production of functional processed cheese supplemented with nanoliposomes of mandarin peel extract. **Materials and Methods:** The encapsulation efficiency (EE) of mandarin peel extract powder (MPEP) was examined at five concentrations (0.2, 0.4, 0.6, 0.8 and 1% w/v) and inclusion the highest EE in processed cheese by replacing water with MPEP nanoliposomes at ratios 25, 50 and 100% v/v. The physicochemical properties and phenolics content for processed cheese were analyzed. **Rustles:** High EE (>80%) of MPEP nanoliposomes was achieved. The chemical composition of the resultant processed cheese was in accordance with the Egyptian standard for half fat processed cheese. Physical and organoleptic properties and color parameters of processed cheese supplemented with MPEP nanoliposomes inferior to the control. **Conclusion:** The characterizations of processed cheese samples supplemented with MPEP nanoliposomes remained unaffected during cold storage. The MPEP nanoliposomes were effectively retained within processed cheese, presented a simple and effective delivery vesicle for phenolic compounds.

Key words: Processed cheese, nanoliposomes, mandarin peel extract, phenolic compounds

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Corresponding Author: Marwa Mohamed El-Said, Department of Dairy Sciences, National Research Centre, Giza, Egypt Tel: 00201018511447

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

Healthy food has increased depending on the consumer's demand which due to develop food products supplemented with bioactive compounds especially polyphenols, originating from natural sources[1]. Dried peels of mature mandarin (*Citrus reticulate*) are a rich source of many bioactive compounds such as; polyphenols, carotenoids, vitamins, enzymes and dietary fibers[2]. Also, mandarin peel has a potent antioxidant and anti-inflammatory activities[3] and is considered a potential source of functional components[4]. Although these valuable components have beneficial characterizations, peels are being discarded. Processed cheese is a good source of nutritional value, ubiquitous consumption and long shelf-life[5]. The supplementation of processed cheese with bioactive components has increased in recent years. The result of the combination of essential oils extracts; medicinal herbs into cheese ameliorated the nutritional value, sensory attributes and reduce the deterioration process of quality parameters in various cheese[6].

As a result of the interactions between phenolic compounds and milk proteins this considered the major reason for the loss of phenolic antioxidant activity[7]. To alleviate the loss activity, a simple and rapid liposomal encapsulation method was used to trap and protect phenolic compounds in mandarin peel from interacting with milk proteins[8]. Up till now, the information about supplementation of processed cheese with bioactive compounds is still limited.

Therefore, the aim of the present study was production of functional food (processed cheese) supplemented with mandarin peel extract (MPE) in nanoliposomes also studied the effect of MPE nanoliposomes on the characterizations of processed cheese samples during cold storage (3 months).

MATERIALS AND METHODS

Materials: This study was made in National Research Centre (NRC), Cairo, Egypt on April, 2018. Egyptian Mandarin fruit (Clementine type) was bought from a local market in Cairo; Egypt. Fresh raw buffalo milk was brought from Faculty of Agriculture, Cairo University, Egypt. Cheddar cheese, Ras cheese and Butter obtained from Cairo market. Low heat skim milk powder was purchased from Irish Dairy Board, Grattan House, Ireland. Calf rennet powder (Ha-La) and whey protein powder were obtained from CHR-Hansen's Lab., Denmark. Unsalted butter was gained from Dina farm, Sadat, Egypt. Commercial emulsifying salt K-2394 (Rhone-Poulenc Chimie, France) were obtained from International Dairy and Foods Co., 10th Ramadan, Egypt. Soy lecithin (69.3% phosphatidyl choline, 9.8% phosphatidyl ethanol amine and 2.1% lysophosphatidylcholine) was provided by Lipoid AG (Ludwigshafen, Germany). Sodium acetate and acetic acid glacial purchased from Carl Roth GmbH and Co., KG (Karlsruhe, Germany). Folin-Ciocalteu reagent, gallic acid and 1, 1-diphenyl-2- pycrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louis, USA). All the solvents for extraction, HPLC analysis and chemicals for antioxidant assays were purchased from Sigma-Aldrich (Tedia Company, USA).

Methods

Preparation of mandarin peel powder: Mandarin fruits were washed by tap water then peeled and dried at room temperature and ambient humidity. After drying, peels were grinded into a fine powder.

Preparation an aqueous extract of mandarin peel: Mandarin peel powder was extracted as described by El-Said *et al.*[9]. Mandarin peels powder (2, 4, 6, 8 and 10%) was extracted in water phase at 40°C for 15, 30 and 60 min under stirring; the same concentrations were soaked also in water phase for 24 h. Each concentration was filtrated using filter paper Whatman No. 1. The filtrate was placed in dark bottles and stored in -18°C until used.

Identification of phenolic and flavonoid compounds in mandarin peel: High-performance Liquid Chromatography Measurement (HPLC) analysis was carried out using an Agilent 1260 series. The separation was carried out using a C18 column (4.6 mm×250 mm i.d., 5 µm). The mobile phase consisted of water: 0.02% tri-floro-acetic acid in acetonitrile (80:20) at a flow rate 1 mL min^{-1}. The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µL for each of the sample solutions. The column temperature[10] was maintained at 35°C.

Determination of antioxidant activity (AA): The antioxidant activity was determined by the ability of antioxidants to scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a free radical[11]. The antioxidant activity was calculated by using the following equation:

$$\text{Antioxidant Activity (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right)\times 100$$

Determination of phenolic compounds (PC): The PC was determined according to previously published method[12] using Folin-Ciocalteu reagent. The results were expressed as milligrams equivalent gallic acid per gram of dry weight.

Freeze-drying of mandarin peel extract powder (MPEP): The MPEP was freeze-dried using Lab conco, USA, at -52°C for 48 h at a pressure below 0.1 MPa. Finally, dried content was grinded to fine powder and kept at -18°C until analyzed.

Preparation of multilayered liposomes

Preparation of primary liposomes: Lecithin powder (2%) was dissolved in 100 mL acetate buffer (0.1M, pH 3.5±0.1) and stirred overnight to ensure its dissolution and homogeneity. Various concentrations of MPEP (0.2, 0.4, 0.6, 0.8 and 1%) were added to the lecithin solutions.

Preparation of secondary liposomes using a chitosan layer: Chitosan was prepared in acetate buffer (0.1M, pH 3.5±0.1) (0.8% w/v). The MPEP liposomes were added to the chitosan solution (1:1) and were stirred by a vortex for preparation of the secondary liposomes.

Preparation of tertiary liposomes using a maltodextrin layer: Chitosan-coated liposomes were dialyzed with dialysis tubes in order to coat the second layer. Secondary liposomes were dialyzed against acetate buffer (0.1M, pH 3.5±0.1) for three days. The dialyzed samples were diluted (1:10) and were added to test-tubes containing maltodextrin solution (20% w/v). The tertiary liposomes, maltodextrin were used to coat of secondary liposomes (1:1).

Characterizations of MPEP nanoliposomes

Measurements of particle size distribution and zeta potential: The particle size and zeta potential were determined with a dynamic light scattering instrument (Nano ZS, Malvern Instruments and Worcestershire, UK). The refractive index for the lecithin was 1.37±0.02. The liposomal solutions were diluted to concentrations of 0.1% (w/w) before the measurement.

Transmission electron microscope (TEM): Twenty micro liters of diluted samples were placed on a film-coated 200-mesh copper specimen grid for 10 min and the excess fluid was removed using filter paper. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope (JEM-2100 Electron Microscope). The samples were observed by operating[13] at 160 kV.

Encapsulation efficiency (EE): The dialysis tube diffusion technique was used to determine EE[9]. Aliquot of 5 mL of the liposome suspension was placed in the dialysis tube, closed tightly and dialyzed against the acetate buffer at 37°C under continues stirring. Samples were taken from the dialysated at success intervals and assayed.

Table 1: Chemical composition of ingredients used in processed cheese

Ingredients	Total solids (%)	Fat (%)	Crude protein (%)	Ash (%)
Cheddar cheese	66.00	35.00	26.03	05.45
Ras cheese	54.91	24.83	22.36	05.85
Skim milk powder	96.00	00.97	37.15	07.85
Butter	84.00	82.00	ND	ND

Manufacture of processed cheese: Firstly, cheddar cheese and Ras cheese were cut into small pieces (approx. 2×2×2 cm) and put into placed in the processing kettle (Stephans universal machine, Switzerland) of 2.5 kg capacity and minced for 30 sec. Subsequently, the mixture of emulsifying salts, butter, skim milk powder, water and MPEP nanoliposomes which replaced water with different ratios of MPEP nanoliposomes 25, 50 and 100% were heated up at 80°C with continues mixing for 10 min. Finally, samples were poured into 100 mL sterilized glass jar with sealable lids. The packed samples were cooled down rapidly and stored 6±2°C until the analyses was performed[14]. Table 1 showed the chemical composition (%) of the above ingredients.

Chemical analysis: Total solids, fat, protein, ash and salt were determined according to methods described by AOAC[15]. Lactose content was determined calorimetrically using phenol-sulphuric acid method as described by Barnett and Tawab [16]. The pH was measured using pH meter (JENWAY 3505) equipped with combined electrode.

Physical analysis

Penetration: The firmness of cheese samples were determined using a penetrometer supplied by Koehler instrument Company Inc., 1595 Sycamore Avenue, Bohemio, New York 11716, USA. A cone assembly weighted 35 g and the depth of penetration was measured in 1/10 mm and in general the greater the depth of penetration the weaker the body of cheese. The test was performed as follows: The penetrometer cone was adjusted to touch the surface of PCSs sample then; the cone was released to penetrate the sample for 5 sec. The penetration depth was recorded in units of 0.1 mm penetrometer reading in related inversely to the firmness of cheese samples.

Oil separation: Oil separation was determined according to the method outlined by Thomas[17].

Meltability: Meltability of cheese samples was determined according to the method designed in previous study[18] as modified by Savello *et al.*[19].

Color measurement: Hunter LAB (Color quest XE, Hunter Lab, USA) was used for analyzing the color of yoghurt samples. Illuminant D65 was the source of light and the observation was 10°. The color value evaluated as L* (Lightness), a* (negative value means green and positive value means red) and b* (negative value means blue and positive value means yellow). All samples were done triplicate.

Sensory evaluation: All samples of processed cheese were evaluated organoleptically for the different sensory properties provided already[20]. Cheese scoring was carried out by a score panel of 12 personal of the staff members of the Dairy Department of the National Research Centre.

Experimental: Formulations of the different treatments (3 treatments) are shown in Table 2. Three replicates were made from each treatment and analyzed each in duplicate.

Table 2: Formulation of ingredients (g) used in the preparation of processed cheese

Ingredients	Control	T_1*	T_2**	T_3***
Cheddar cheese	15.86	15.86	15.86	15.86
Ras cheese	47.58	47.58	47.58	47.58
Butter	07.61	07.61	07.61	07.61
Emulsifier SOH	02.13	02.13	02.13	02.13
Water	26.82	20.12	13.41	0.00
MPEP liposomes	0.00	6.70	13.41	26.82

Control: Processed cheese without MPEP nanoliposomes, T_1*: Processed cheese with 25% of MPEP nanoliposomes, T2**: Processed cheese with 50% of MPEP nanoliposomes, T_3***: Processed cheese with 100% of MPEP nanoliposomes

Statistical analyses: The data obtained in this study were expressed as the mean of triplicate determinations. Statistical comparisons were made with Duncan's test which was analyzed with SPSS for Windows, Version Rel. 10.0.5., Inc. $p<0.05$ were considered to be significant[21].

RESULTS

Identification of phenolic and flavonoid compounds by HPLC: A total of 11 phenolic compounds-including six phenolic acids and five flavonoids were identified and quantified in mandarin peel (Fig. 1). Total amount of phenolic compounds was 3896.73 μg g^{-1} of mandarin peel (Table 3). Gallic acid and catechin were highly concentration in mandarin peel, whereas, cinnamic acid and propyl gallate were the least quantified phenolic compounds.

Table 3: Phenolic compounds (μg g^{-1}) in MPEP

Phenolic compounds	Concentration (μg g^{-1})
Gallic acid	1774.54
Catechin	1426.38
Caffeic acid	68.52
Syringic acid	20.78
Rutin	120.91
Coumaric acid	332.47
Ferulic acid	66.02
Quercetin	64.64
Cinnamic acid	02.98
Propyl gallate	05.28
4.7-Dihydroxyisoflavone	14.21
Total amount of phenolic compounds	3896.73

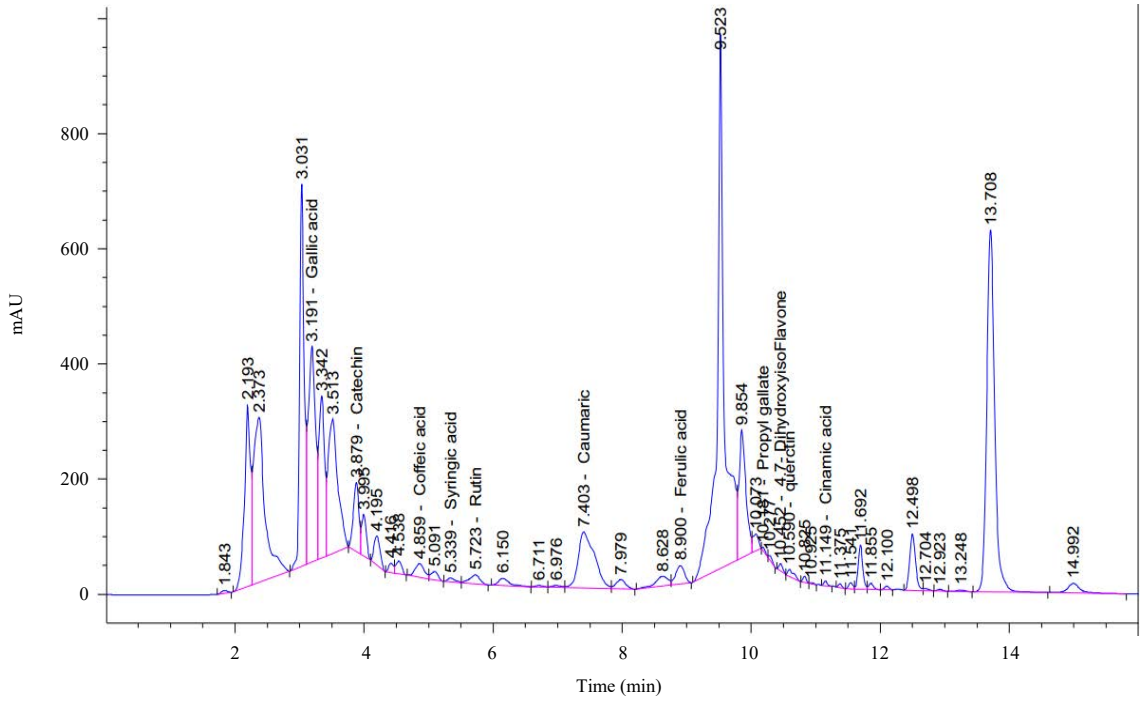

Fig. 1: HPLC of phenolic and flavonoid compounds in MPEP

Table 4: Antioxidant activity (AA) (%) of MPEP with different treatments

	AA (%)			
	40°C			
Concentrations of MPEP (%)	15 min	30 min	60 min	Socking/24 h
2	32.77±0.005^{d}	41.60±0.051^{c}	45.66±0.009^{d}	39.50±0.018^{d}
4	57.60±0.009^{c}	65.07±0.000^{b}	68.90±0.048^{c}	56.59±0.012^{c}
6	75.84±0.026^{b}	70.44±0.017^{ab}	72.10±0.012^{c}	74.89±0.032^{b}
8	78.48±0.011^{b}	73.44±0.008^{a}	77.77±0.044^{b}	77.73±0.003^{ab}
10	85.76±0.000^{a}	74.55±0.016^{a}	83.58±0.008^{a}	80.29±0.005^{a}

Different letters in the columns represent statistically significant differences ($p<0.05$)

Table 5: PC of MPEP with different treatments

	PC (Equivalent mg gallic acid g^{-1} DW)			
	40°C			
Concentrations of MPEP (%)	15 min	30 min	60 min	Socking/24 h
2	225.40±0.001^{d}	221.05±0.003^{d}	213.60±0.000^{d}	211.60±0.011^{e}
4	355.50±0.014^{c}	354.20±0.019^{c}	329.90±0.012^{c}	337.00±0.005^{d}
6	462.75±0.008^{b}	427.80±0.011^{b}	414.20±0.021^{b}	420.50±0.005^{c}
8	489.80±0.021^{b}	477.90±0.033^{a}	451.65±0.004^{a}	479.65±0.020^{b}
10	527.30±0.005^{a}	502.05±0.010^{a}	462.05±0.002^{a}	527.85±0.004^{a}

Different letters in the columns represent statistically significant differences ($p<0.05$)

Antioxidant activity (AA) of MPEP: Antioxidant activity (AA) of the MPEP extracts were evaluated via 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity. The effect of mandarin peel concentration and extraction time on the yield of antioxidant activity in the aqueous extract was shown in Table 4. It can be seen that the highest results concerning the AA was obtained using 10% mandarin peel at 40°C for 15 min also, any increase in the temperature extract (after 40°C) didn't give significantly increase in AA in the extract.

Phenolics content (PC) of MPEP: Results from spectrophotometric determination of phenolics content in MPEP extracts are summarized in Table 5 as mg of gallic acid equivalent per gram DW and as the content of phenolic compounds in the extract. Mean values of PC are represented as the mean taking into account the standard deviation. The highest value of PC was observed when MPEP used at 10% at 40°C for 15 min also, any increase in the temperature extract (after 40°C) didn't give significantly increase in PC values in the extract. This indicated that the extract reached to the maximum solubility of phenolic compounds under these conditions.

Characterizations of MPEP nanoliposomes: The microencapsulation efficiency (EE) of MPEP nanoliposome was shown in Fig. 2. The EE was 70.41, 73.20, 73.92, 82.29 and 83.90% for 0.2, 0.4, 0.6, 0.8 and 1% of MPPE in nanoliposome, respectively. It was found that there is no significant difference in EE between 0.8 and 1% of MPPE ratios in liposome.

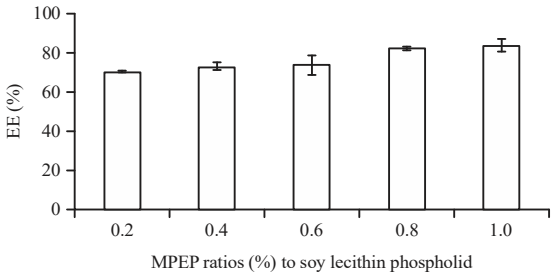

Fig. 2: Encapsulation efficiency (EE) (%) of MPEP nanoliposomes with different ratios of MPEP to soy lecithin phospholipids

The particle size average diameters and zeta-potentials of MPEP nanoliposomes at different ratios are presented in Fig. 3. It was found from the results, increasing the concentration of MPPE liposomes caused an increment of the particle size. The z-potentials of the liposomes containing 0.8% MPEP (approximately -21.55 mV) did not change as much as those of liposome containing 1% (-23.25 mV). The results indicated that MPEP nanoliposomes had a narrow particle-size range (250-450 nm) with a relatively uniform distribution and all particles distributed in less than 1000 nm.

The exemplary images of MPEP nanoliposomes containing 0.8% MPEP is shown in Fig. 4. The observed liposomes displayed a spherical shape and no aggregates could be visually detected in the samples.

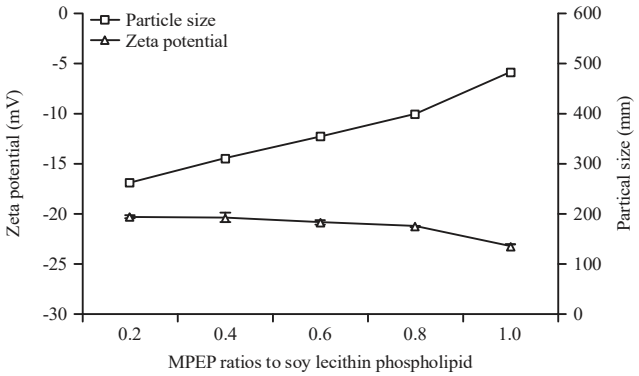

Fig. 3: Particle size and zeta potential of MPEP nanoliposomes with different ratios of MPEP to soy lecithin phospholipids

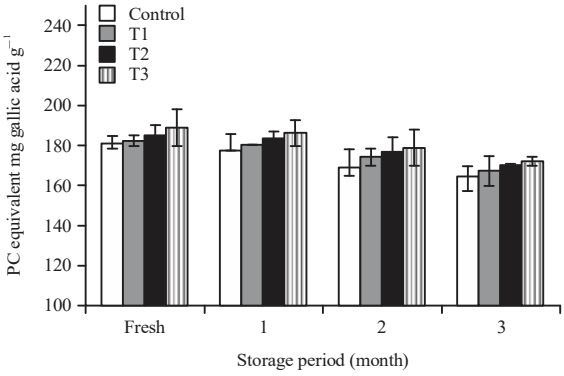

Fig. 5: Phenolics content (PC) of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

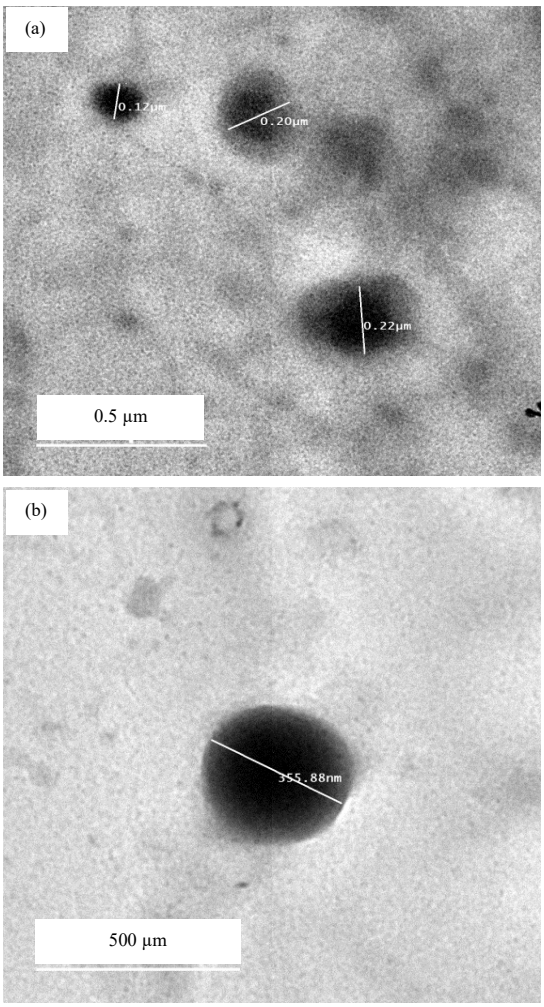

Fig. 4(a-b): TEM micrograph of MPEP nanoliposomes with 0.8% MPEP obtained at a scale of 0.5 µm (500 nm)

Characterizations of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

Phenolics content (PC): The PC of cheese samples measured over the 90 days during the cold storage was shown in Fig. 5. In spite of the addition of MPEP nanoliposomes to cheese formula, it hadn't affected the PC values of cheese samples ($p<0.05$) while the mean values of PC increased steadily with ripening time in control or treated cheese samples.

Chemical composition: Based on the chemical composition (total solids, protein, fat, lactose and salt in moisture) of cheese samples Table 6, there wasn't any significant difference ($p<0.05$) observed among different constituents between control and treated cheese samples (T_1, T_2 and T_3). Also, from the results, there weren't any significant differences in pH values were found among the control and treated cheeses observed over the 90 days ripening period.

Physical properties: The physical properties of processed cheese supplemented with different ratios of MPEP nanoliposomes (T_1, T_2 and T_3) are shown in Table 7. It was clear from Table 7, the mean value of penetration for control sample had the highest value and these values decreased with increasing MPEP addition (control>T_1>T_2>T_3) at fresh while these values were decreased during storage for control and cheese treated. There isn't any significant difference in oil separation values between control and treated cheese samples (T_1, T_2 and T_3) at fresh. Moreover, oil separation values increased with increasing addition of MPEP nanoliposomes (control>T_1>T_2>T_3), while the oil separation values increased during the cold storage period for all samples, being slightly in control sample than the treated cheese samples. The

Table 6: Chemical composition of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

Chemical composition	Control	T_1	T_2	T_3
Total solids	44.97±0.02[b]	44.97±0.03[b]	45.01±0.01[b]	45.07±0.03[a]
Protein	13.71±0.01[a]	13.70±0.02[a]	13.72±0.01[a]	13.71±0.01[a]
Fat/DM	50.05±0.02[a]	50.04±0.01[a]	50.03±0.03[a]	50.02±0.03[a]
Lactose	01.10±0.01[a]	01.10±0.01[a]	01.08±0.01[a]	01.09±0.01[a]
Salt in moisture	03.66±0.01[a]	03.64±0.02[a]	03.64±0.03[a]	03.62±0.03[a]
pH	05.76±0.01[a]	05.76±0.01[a]	05.71±0.01[b]	05.71±0.01[b]

Different letters in the columns represent statistically significant differences ($p<0.05$). Control: Processed cheese without MPEP nanoliposome, T1: Processed cheese with 25% of MPEP nanoliposome, T2: Processed cheese with 50% of MPEP nanoliposome, T3: Processed cheese with 100% of MPEP nanoliposome

Table 7: Physical properties of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

Parameters	Storage period	Control	T_1	T_2	T_3
Penetration (mm)	Fresh	175.45±0.19[a]	173.62±0.70[a]	173.60±0.54[a]	171.46±0.45[a]
	1 Month	170.28±0.39[b]	170.25±0.35[b]	169.18±0.25[b]	169.11±0.15[b]
	2 Month	165.42±0.59[c]	164.17±0.23[c]	163.11±0.15[c]	162.23±0.33[c]
	3 Month	163.36±0.50[d]	161.10±0.13[d]	161.15±0.21[d]	160.31±0.44[d]
Oil separation (%)	Fresh	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
	1 Month	20.55±0.57[b]	21.33±0.46[b]	20.60±0.49[b]	21.23±0.32[b]
	2 Month	21.23±0.32[b]	21.33±0.25[b]	21.39±0.39[b]	21.41±0.13[b]
	3 Month	22.51±0.21[a]	22.89±0.11[a]	22.90±0.09[a]	23.24±0.33[a]
Melting index (mm)	Fresh	115.51±0.51[a]	112.36±0.26[a]	111.58±0.41[a]	107.07±0.87[a]
	1 Month	112.49±0.36[b]	110.37±0.40[b]	102.05±0.37[b]	99.87±0.30[b]
	2 Month	96.33±0.20[c]	94.88±0.30[c]	94.74±0.61[c]	94.54±0.65[c]
	3 Month	91.67±0.47[d]	90.56±0.62[d]	90.12±0.17[d]	88.56±0.78[d]

Different letters in the columns represent statistically significant differences ($p<0.05$). Control: Processed cheese without MPEP nanoliposome, T1: Processed cheese with 25% of MPEP nanoliposome, T2: Processed cheese with 50% of MPEP nanoliposome, T3: Processed cheese with 100% of MPEP nanoliposome

Table 8: Color parameters of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

Color parameters	Storage period	Control	T_1	T_2	T_3
L	Fresh	88.08±0.10[a]	85.67±0.18[a]	83.42±0.43[a]	79.33±0.46[a]
	1 Month	87.46±0.33[a]	84.24±0.18[b]	81.58±0.39[b]	82.98±5.98[a]
	2 Month	86.13±0.18[b]	82.53±0.52[c]	80.47±0.37[c]	77.61±0.42[a]
	3 Month	85.52±0.61[b]	79.39±0.30[d]	79.09±0.04[d]	77.34±0.20[a]
a	Fresh	-2.28±0.04[a]	1.17±0.08[b]	1.85±0.13[a]	2.27±0.06[a]
	1 Month	-2.42±0.04[b]	1.15±0.03[b]	1.77±0.06[a]	2.32±0.10[a]
	2 Month	-2.65±0.06[c]	1.27±0.03[b]	1.83±0.04[a]	2.34±0.05[a]
	3 Month	-2.80±0.12[d]	1.65±0.05[a]	1.85±0.06[a]	2.39±0.04[a]
b	Fresh	18.25±0.06[d]	21.22±0.05[d]	24.31±0.08[d]	27.38±0.11[d]
	1 Month	19.88±0.04[c]	22.28±0.05[c]	25.87±0.10[c]	28.24±0.11[c]
	2 Month	20.29±0.06[b]	23.17±0.03[b]	26.22±0.05[b]	29.09±0.09[b]
	3 Month	22.76±0.06[a]	23.58±0.04[a]	27.17±0.08[a]	30.14±0.08[a]

Different letters in the columns represent statistically significant differences ($p<0.05$). Control: Processed cheese without MPEP nanoliposome, T1: Processed cheese with 25% of MPEP nanoliposome, T2: Processed cheese with 50% of MPEP nanoliposome, T3: Processed cheese with 100% of MPEP nanoliposome

meltability values of treated cheese samples ($T_1>T_2>T_3$) were lower than the control sample at fresh while, these values decreased as the storage period advanced for all cheese samples. At the end of the storage period, the high concentration of the MPEP nanoliposomes (T_3) showed a significant meltability effect ($p<0.05$).

Color parameters: The color parameters of processed cheese supplemented with different ratios of MPEP nanoliposomes were shown in Table 8. It is obvious from these results, at fresh all cheese samples had more whiteness degree (L) compared to during storage period. The control sample had a higher whiteness degree than the treated cheese ($T_1>T_2>T_3$) at fresh and in during storage periods. However, the control sample had a green color degree (a) more than the treated cheese which green color decreased with increasing addition of MPEP nanoliposomes concentration. Also, the increase of parameter b (yellow degree) was highly pronounced in treated cheese ($T_3>T_2>T_1$) than the control sample at fresh and during storage period. This increase may be due to the slightly yellow color of added MPEP nanoliposomes.

Table 9: Organoleptic attributes of processed cheese supplemented with different ratios of MPEP nanoliposomes during cold storage

Character assessed	Control	T_1	T_2	T_3
Appearance	9.35±0.21^{a}	9.45±0.21^{a}	9.45±0.07^{a}	9.55±0.07^{a}
Flavor	8.85±0.21^{a}	8.60±0.14^{b}	8.45±0.07^{c}	8.30±0.14^{d}
Firmness	8.70±0.14^{a}	8.40±0.14^{ab}	8.10±0.14^{bc}	7.95±0.07^{c}
Spreading	8.25±0.07^{c}	8.45±0.07^{b}	8.65±0.07^{a}	8.67±0.06^{a}
Stickiness	7.35±0.21^{a}	6.95±0.07^{a}	6.35±0.21^{b}	6.30±0.14^{b}
Crumbliness	6.80±0.14^{a}	6.35±0.21^{b}	6.10±0.14^{b}	6.00±0.00^{b}
Overall acceptability	8.35±0.35^{a}	8.10±0.14^{a}	7.65±0.35^{ab}	7.15±0.21^{b}

Different letters in the columns represent statistically significant differences ($p<0.05$).Control: Processed cheese without MPEP nanoliposome, T1: Processed cheese with 25% of MPEP nanoliposome, T2: Processed cheese with 50% of MPEP nanoliposome, T3: Processed cheese with 100% of MPEP nanoliposome

Organoleptic properties: Organoleptic properties of processed cheese samples were carried out at fresh, as well as every 30 days up to the end of storage period of 90 days. The score of appearance, flavor, firmness, spreading, stickiness and crumbliness of processed cheese were shown in Table 9. The score of cheese appearance showed that T_3 had a highest value then T_1 and T_2 were similar and the lowest value was control sample. Score of appearance of all processed cheese tended to decrease with the advance of storage period. Flavor, firmness, stickiness and crumbliness scored high values in the control sample then T_1 and T_2 and finally T_3 scored the lowest value. Concerning the overall acceptability, most of the panelists preferred the control and MPEP nanoliposomes samples. Overall, based on data collected from sensory evaluation in this study, adding MPEP nanoliposomes into processed cheese resulted in close consumer scores to control sample. Concerning the overall acceptability, most of the panelists preferred the control and MPEP nanoliposomes samples. Overall, based on data collected from sensory evaluation in this study, adding MPEP nanoliposomes into processed cheese resulted in close consumer scores to control sample.

DISCUSSION

Dried mandarin peel (*Citrus reticulate*) is a rich source of polyphenols, especially gallic and catechin had in a high concentration in all fruit peels along with rutin and quercetin. Also, it is clear from these results, whenever increased the concentration of mandarin peels, increased phenolics content and the antioxidant activity in the extract, but it was observed increasing extraction time decreased phenolics content and the antioxidant activity. Decreasing phenolic compounds and antioxidant activity in the extract as a result of increasing temperature time may due to the degradation of phenolic compounds due to their stable structure features, thermal instability and these data agree with Farrag *et al.*[22]. Also, the fruit peels may contain higher concentrations of phenolic compounds because they are in the external part of the fruit, so, they are more exhibits to the synthesis of phenolic compounds[23].

In the liposomal structure, the aqueous core and bilayer wall were the hydrophilic and hydrophobic parts, respectively, therefore, the phospholipid bilayers acted as the reservoir for phenolics. The MPEP has not only integrated into the inner of the liposome, but also partially integrated into the phospholipid membrane or had been adsorbed onto the surface of liposomes. It is generally accepted that the encapsulation efficiency of the active substances within liposomal structure can be affected by the size and/or specific surface areas of the liposomes[24]. However, exceeding 0.8% of MPEP concentration, there isn't any significant effect on particle size. It means that after this concentration, excess MPEP was not integrated into the liposome structure.

The size of MPEP nanoliposomes were within the size range reported by other studies[25].

Meantime, the zeta potential of MPEP nanoliposomes a ranged between -20.30 and -23.25 mV since negatively charged of MPEP nanoliposomes enhanced the negativity of liposomes and these results confirmed the negative surface charges on the MPEP nanoliposomes due to maltodextrin layer (negative charge) in tertiary liposomes. Consequently, oppositely charged surfaces were attracted to each other and formed aggregates. For these reasons, the particle diameters increased. The strongly negative z-potential of 1% MPEP in liposome was caused by oligomeric polyphenolic compounds. These results are appropriate for our previous study[9].

The PC values of the treated cheese samples supplemented with MPPE nanoliposomes especially after ripening were higher than the control cheese samples. The control samples had a significant amount of phenolic compounds due to the endogenous phenolic compounds in milk, derived from proteins (such as; tyrosine residues), sugar components, oligosaccharides and lactose[26]. This result consistent with our previous findings[5] and other reports[27]. The evidence on encapsulation of polyphenols can be considered as a way to preserve the antioxidative potential of these bioactive compounds[28].

In the present study, it was found from the results of adding MPPE nanoliposomes on the total solids, fat, protein, lactose contents and pH, these results are compatible with previous findings for the addition of rosemary extract to cheese by direct dissolution in aqueous solutions[6]. Also, the moisture content and soluble nitrogen of low-fat UF cheese increased with increasing rosemary extract concentrations which contained polyphenols[6]. Also, they mentioned that the changes in the chemical compositions of treated cheese samples caused by the addition of polyphenols have been due to the interactions between phenolic compounds and milk proteins[29]; such interactions may be induced by dissolution of phenolic compounds in milk.

An overall look to the physical properties and organoleptic attributed of processed cheese supplemented with different ratios of MPEP nanoliposomes, it could be recommended that processed cheese could be supplemented with MPPE liposomes up to T_3 and kept at 5°C to get acceptable and high potential health product.

CONCLUSION

This study presented a simple and feasible approach to deliver MPEP in nanoliposomes entrapped in processed cheese system, a food product that is widely consumed and is a nutritious vehicle for protein and calcium in the human diet. Liposome characterizations revealed the lamella structure and central core of liposomes and confirmed the integrity, stability, high encapsulation efficiency (>80%) of the resultant liposomes. Soy lecithin liposomes have shown potential to create functional foods with high phenolic content. The physical, chemical properties and phenolics content of processed cheese supplemented with MPEP nanoliposomes remained unaffected during cold storage of 3 months. Encapsulated MPEP in liposomes is a promising technique to protect and deliver phenolics to the gut.

SIGNIFICANCE STATEMENT

The study discovered the importance of using nanoliposome as encapsulation technique that can be beneficial for overcoming the problem of fortification dairy products with bioactive compounds which may interact with milk proteins, thus reducing the nutritional value of these compounds.

REFERENCES

1. Socaci, S.A., D.O. Rugină, Z.M. Diaconeasa, O.L. Pop and A.C. Fărca *et al*., 2017. Antioxidant Compounds Recovered from Food Wastes. In: Functional Food-Improve Health Through Adequate Food, Hueda, M.C. (Ed.)., IntechOpen, China.
2. Ajila, C.M., K. Leelavathi and U.J.S. Prasada Rao, 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J. Cereal Sci., 48: 319-326.
3. Murakami, A., Y. Nakamura, Y. Ohto, M. Yano and T. Koshiba *et al*., 2000. Suppressive effects of citrus fruits on free radical generation and nobiletin, an anti-inflammatory polymethoxyflavonoid. BioFactors, 12: 187-192.
4. Schieber, A., F.C. Stintzing and R. Carle, 2001. By-products of plant food processing as a source of functional compounds-recent developments. Trends Food Sci. Technol., 12: 401-413.
5. Buňka, F., J. Hrabě and S. Kráčmar, 2004. The effect of sterilisation on amino acid contents in processed cheese. Int. Dairy J., 14: 829-831.
6. Hala, M.F.E., I.G. Ebtisam, M.A.B. Sanaa, A.S. Gad and M.E. Marwa, 2010. Manufacture of low fat UF-soft cheese supplemented with rosemary extract (as natural antioxidant). J. Am. Sci., 6: 570-579.
7. Mehanna, N.S., Z.M.R. Hassan, H.M.F. El-Din, A.A.E. Ali, R. Amarowicz and T.M. El-Messery, 2014. Effect of interaction phenolic compounds with milk proteins on cell line. Food Nutr. Sci., 5: 2130-2146.
8. Rashidinejad, A., E.J. Birch, D. Sun-Waterhouse and D.W. Everett, 2014. Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. Food Chem., 156: 176-183.
9. El-Said, M.M., T.M. El-Messery and H.M.F. El-Din, 2018. The encapsulation of powdered doum extract in liposomes and its application in yoghurt. Acta Scient. Pol. Technol. Aliment., 17: 235-245.
10. Kelebek, H., A. Canbas and S. Selli, 2008. Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. Food Chem., 107: 1710-1716.
11. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
12. Jayaprakasha, G.K., R.P. Singh and K.K. Sakariah, 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73: 285-290.

13. Saloko, S., P. Darmadji, B. Setiaji, Y. Pranoto and A.K. Anal, 2013. Encapsulation of coconut shell liquid smoke in chitosan-maltodextrin based nanoparticles. Int. Food Res. J., 20: 1269-1276.
14. Mehanna, N.S., F.A.M. Hassan, T.M. El-Messery and A.G. Mohamed, 2017. Production of functional processed cheese by using tomato juice. Int. J. Dairy Sci., 12: 155-160.
15. AOAC., 2007. Official Method of Analysis. 18th Edn., Association of Official Analytical Chemist, Gaithersburg, MD., USA.
16. Barnett, A.J.G. and G.A. Tawab, 1957. A rapid method for the determination of lactose in milk and cheese. J. Sci. Food Agric., 8: 437-441.
17. Thomas, M.A., 1973. The use of hard milk fat fraction in processed cheese. Aust. J. Dairy Technol., 28: 77-83.
18. Olson, N.F. and W.V. Price, 1958. A melting test for pasteurized process cheese spreads. J. Dairy Sci., 41: 999-1000.
19. Savello, P.A., C.A. Ernstrom and M. Kalab, 1989. Microstructure and meltability of model process cheese made with rennet and acid casein. J. Dairy Sci., 72: 1-11.
20. Cunha, C.R., A.I. Dias and W.H. Viotto, 2010. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. Food Res. Int., 43: 723-729.
21. SPSS, 1999. SPSS for Windows User's Guide Release 10. SPSS Inc., Chicago.
22. Farrag, A., T.M. El-Messery, M.M. El-Said, T.N. Soliman and H.M.F. El-Din, 2018. Microencapsulation of grape phenolic compounds using whey proteins as a carrier vehicle. J. Biol. Sci., 18: 373-380.
23. Ko, S.H., J.H. Park, S.Y. Kim, S.W. Lee, S.S. Chun and E. Park, 2014. Antioxidant effects of spinach (*Spinacia oleracea* L.) supplementation in hyperlipidemic rats. Prevent. Nutr. Food Sci., 19: 19-26.
24. Rasti, B., S. Jinap, M.R. Mozafari and A.M. Yazid, 2012. Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. Food Chem., 135: 2761-2770.
25. Zou, L.Q., W. Liu, W.L. Liu, R.H. Liang and T. Li *et al*., 2014. Characterization and bioavailability of tea polyphenol nanoliposome prepared by combining an ethanol injection method with dynamic high-pressure microfluidization. J. Agric. Food Chem., 62: 934-941.
26. Ignat, I., I. Volf and V.I. Popa, 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem., 126: 1821-1835.
27. Gibis, M., E. Vogt and J. Weiss, 2012. Encapsulation of polyphenolic grape seed extract in polymer-coated liposomes. Food Funct., 3: 246-254.
28. Dang, S., S. Gupta, R. Bansal, J. Ali and R. Gabrani, 2015. Nano-Encapsulation of a Natural Polyphenol, Green Tea Catechins: Way to Preserve its Antioxidative Potential. In: Free Radicals in Human Health and Disease, Rani, V., U. Chand and S. Yadav (Eds.)., Springer, New Delhi, pp: 397-415.
29. Rashidinejad, A., E.J. Birch, D. Sun-Waterhouse and D.W. Everett, 2015. Total phenolic content and antioxidant properties of hard low-fat cheese fortified with catechin as affected by *in vitro* gastrointestinal digestion. LWT-Food Sci. Technol., 62: 393-399.