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Enhancement of the Stability of Encapsulated Pomegranate (*Punica granatum* L.) Peel Extract by Double Emulsion with Carboxymethyl Cellulose

Essam Hady [1], Mahmoud Youssef [1], Amani H. Aljahani [2], Huda Aljumayi [3], Khadiga Ahmed Ismail [4], El-Sayed El-Damaty [1], Rokkaya Sami [3,*] and Gamal El-Sharnouby [1,*]

1 Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo 11751, Egypt; essamhady@azhar.edu.eg (E.H.); mahmoudyoussef@azhar.edu.eg (M.Y.); damatyelsayed@yahoo.com (E.-S.E.-D.)
2 Department of Physical Sport Science, College of Education, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; ahaljahani@pnu.edu.sa
3 Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; huda.a@tu.edu.sa
4 Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; khadigaah.aa@tu.edu.sa
* Correspondence: rokayya.d@tu.edu.sa (R.S.); gamali59@azhar.edu.eg (G.E.-S.)

Abstract: Pomegranate peel enriched with high value of bioactive phenolics with valuable health benefits. However, after extraction of the phenolic compounds, diverse factors can affect their stability. Therefore, we, herein, aimed to prepare $W_1/O/W_2$ double nanoemulsions loaded with phenolic-rich extract from pomegranate peel in the W_1 phase. Double emulsions were fabricate during a two-step emulsification technique. Furthermore, the influence of sodium carboxymethyl cellulose (CMC) in the outer aqueous phase was also investigated. We found that $W_1/O/W_2$ emulsions containing phenolic-rich extract showed good physical stability, especially in the particle size, polydispersity index, zeta potential, and creaming index. Intriguingly, high encapsulation rates of pomegranate polyphenols >95% were achieved; however, emulsion with CMC had the best encapsulation stability during storage. Thus, our study provides helpful information about the double nanoemulsions delivery system for polyphenols generated from pomegranate peel, which may lead to the development of innovative polyphenol-enriched functional foods.

Keywords: pomegranate peel; encapsulation; $W_1/O/W_2$ nanoemulsion; phenolic compounds; physical stability; carboxymethyl cellulose

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1. Introduction

Pomegranate (*Punica granatum* L.; Punicaceae) is a fruit-bearing deciduous shrub extensively grown in many areas such as the Middle East, Europe, and Southeast Asia [1]. The global pomegranate production is around 8 M tons [2]. Pomegranate consumption has dramatically risen as people become more aware about its excellent therapeutic properties [3]. It is commonly consumed fresh or in the form of juice. Pomegranate juice manufacturing generates vast amounts of by-products, such as seeds and peels, which cause disposal problems and environmental pollution [4]. Hence, these wastes have received great attention from investigators.

Pomegranate peel accounts for nearly around 30 to 40% of the whole fruit weight [5]. The utilization of pomegranate peel to produce bioactive compounds leads to yield a high value of by-products, and reduces agricultural waste and environmental problems [6]. Pomegranate peel contains large amounts of different secondary metabolites, especially flavonoids, phenols, and hydrolyzable tannins such as catechin, gallic acid, caffeic acid, anthocyanins, and many others [7].

Historically, the ancient Egyptians and Indians used pomegranate peel against various diseases such as cough, diarrhea, dysentery, ulcers, diabetes, cancer, and cardiovascular diseases. This is because of its biological activities, e.g., antioxidant, anti-inflammatory, antihepatotoxic, antigenotoxic, and memory-enhancing characteristics [8–10]. Furthermore, pomegranate peel has proven its beneficial effects in treating diseases caused by free radicals such as aging, wounds, and ulcers [11,12].

Despite the numerous health benefits of pomegranate peel extract (PPE), it is unstable and vulnerable to oxidation, polymerization, and condensation reactions [13]. In addition, phenolic compounds, in general, can be easily destroyed during processing and storage due to its sensitivity to high temperatures, oxygen, and light or when exposed to the gastrointestinal conditions, which restricts their activity and compromises their valuable effects in health [14,15]. Moreover, when pomegranate peel phenolics are incorporated into food, they can cause astringent flavor [16]. Furthermore, the industry currently demands proven methods that permit the using of polyphenols, instead of free molecules for the bioavailability [17]. Consequently, encapsulation of PPE can be an approach not only to improve their stability [18], but also to mask astringency and bitterness [19], and improve their bioavailability and controlled release [20,21].

Thus, emulsion-based encapsulation systems provide an attractive alternative solution to overcome these issues. It is a valuable system to incorporate water-soluble and/or oil-soluble bioactive compounds in functional food products [22].

Double emulsions, in general, can be emulsions inside emulsions. The 1st phase is small spherical droplets dispersed into the 2nd phase to form a primary emulsion, and this primary emulsion is re-dispersed as droplets into the 3rd phase to obtain the double emulsion. There are many types of double emulsions, but the main two types are: water-oil-water ($W_1/O/W_2$) emulsions and oil-water-oil ($O_1/W/O_2$) emulsions. The $W_1/O/W_2$ emulsions have considerable promise for food applications since most foods are constituted of a continuous aqueous phase [23].

Double emulsions display many advantages as follows: 1. Besides their ability to incorporate two types of bioactive compounds together in one system, the double emulsion can be used for targeted delivery of these compounds at a controlled release rate. 2. They can be used to prepare low-fat foods such as salad dressings, fat spreads, and cheeses without affecting the sensory properties. 3. They can be used to deliver functional components by masking their undesirable flavor or odor and protecting sensitive compounds [24].

Recently, $W_1/O/W_2$ emulsions have been used to encapsulate and protect hydrophilic bioactive compounds such as anthocyanin [25], catechin [26], oleuropein [27], and gallic acid [28]. They have also been used to encapsulate phenolic-rich extracts, such as mango peel [29], olive leaf [30], grape seed [31], red dragon fruit [32], or blueberry pomace [33]. To date, encapsulation of PPE in double emulsions has not been studied yet.

The main goal of this study was to prepare $W_1/O/W_2$ double nanoemulsions loaded with pomegranate peel extract (PPE-DE) in the W_1 phase. Non-ionic emulsifiers: "PGPR" and "Tween 20" were used as emulsifiers in W_1–O and O–W_2 interfaces, respectively. The influence of the presence of carboxymethyl cellulose (CMC) in the outer aqueous phase (W_2) was also investigated. The formulated emulsions were characterized based on their physico-chemical properties, namely their microstructures, viscosities, zeta potentials (ζ), mean droplet sizes, droplet size distributions, and Fourier Transform Infrared (FTIR) spectra, in addition to the encapsulation efficiency (EE) of PPE.

2. Materials and Methods

2.1. Materials

Fresh pomegranate fruits (*Punica granatum* L.) Sahrawy Sp. were obtained from the El-Obourmarket (Cairo, Egypt). Corn oil without any added antioxidants (TBHQ -ve) was donated by Arma Food Industries Company, 10th of Ramadan city (Egypt). Gallic acid and Folin–Ciocalteu reagentwere obtained from Sigma-Aldrich (St. Louis, MO, USA); sodium carbonate from Surechem (Suffolk, England). Sodium carboxy methyl cellulose

and Tween 20 were purchased from Advent company, Navi Mumbai (India); polyglycerol polyricinolate (Grinsted PGPR 90 Kosher) was provided from Danisco Company (Copenhagen, Denmark). Other chemicals (analytical grade) were purchased from El-Gamhouria Company (Cairo, Egypt).

2.2. Preparation of Pomegranate Peel Powder

Fresh pomegranate fruits were cleaned up with tap water and dried with a cloth, and seeds were removed. After that, peels were cut using a sharp knife into small pieces around 2 × 2 cm, and then allowed to dry in a laboratory oven with forced air circulation at 45 °C for 24 h. The dried peels were ground into fine powder. Afterwards, the powder was sieved through a 500 µm mesh and kept at −20 °C until analysis.

2.3. Preparation of PPE

To obtain PPE, peel powder (25 g) was extracted with 70% (v/v) EtOH solution (500 mL). The mixture was put in a shaker incubator set at 125 rpm for 24 h in the dark at ambient temperature. The flask was covered with aluminum foil to avoid exposure of light during the extraction process. The extract was filtered through Whatman No.1 filter paper after centrifugation at 3500 rpm for 15 min. A rotary evaporator was used to concentrate the filtered extract at a temperature of 40 °C. Finally, the remaining extract was pre-frozen in −80 °C freezer overnight, and then lyophilized in a freeze dryer at −50 °C for 24 h. The resulting powder was kept at −20 °C for further use.

2.4. Preparations of PPE-DE

As shown in Figure 1, double $W_1/O/W_2$ nanoemulsions were fabricated according to a method previously optimized by Velderrain-Rodriguez et al. [29], with slight changes, as follows: In the first step, primary W_1/O emulsions were comprised of corn oil (70%, wt/wt), the internal water phase (W_1) (22%, wt/wt), glycerol (3%, wt/wt) as cosurfactant, and PGPR (5%, wt/wt) as lipophilic surfactant. Loaded W_1/O emulsions were prepared using the PPE solution (1 mg/mL) dissolved in 0.1 M NaCl solution as W_1, while non-loaded emulsion (Blank, without PPE) was prepared using 0.1 M NaCl solution as W_1. Before emulsification, glycerol and PGPR were dissolved in W_1 and corn oil, respectively, using a magnetic stirrer (60 °C for 5 min). W_1 phase was dispersed in corn oil using a high-speed homogenizer (Unidrive X1000D-CAT, Ballrechten-Dottingen, Germany) operated at 6000 rpm/8 min. Subsequently, to diminish the water droplets' particle size, W_1/O emulsions were sonicated with an Ultrasonic liquid processor (Vibra-cell, VCX-750, Sonics & Materials, Inc., Newtown, CT, USA) for 3 min at 24 kHz frequency and 40% amplitude. In the second step, $W_1/O/W_2$ emulsions were comprised of 25, 73, and 2% (wt/wt) of the W_1/O emulsion, external water phase (W_2), and Tween 20 (as a hydrophilic surfactant), respectively. The W_2 of emulsions was comprised of 0.1 M NaCl solution which was used to dissolve Tween 20 and CMC (0.5% wt/wt). The final $W_1/O/W_2$ emulsions were homogenized with a high-speed homogenizer at 6000 rpm/4 min, and subsequently sonicated for 1.5 min at a frequency of 24 kHz and 30% amplitude.

2.5. Emulsion Characterization

Physical characterization of the double emulsion encapsulating the PPE was performed by measuring mean particle diameter, particle distribution, particle charge via zeta potential, apparent viscosity, and morphological characteristics via transmission electron microscopy (TEM).

2.5.1. Particle Size, Polydispersity Index (PDI), and ζ Potential Measurements

Dynamic light scattering (DLS) (NICOMP 380 ZLS, PSS, Santa Barbara, CA, USA) is a method used to measure particle diameter, PDI, and ζ potential of the prepared emulsions. Samples were analyzed using the 632 nm line of a HeNe laser as the incident light with an angle of 90° and ζ potential with an external angle of 18.9°.

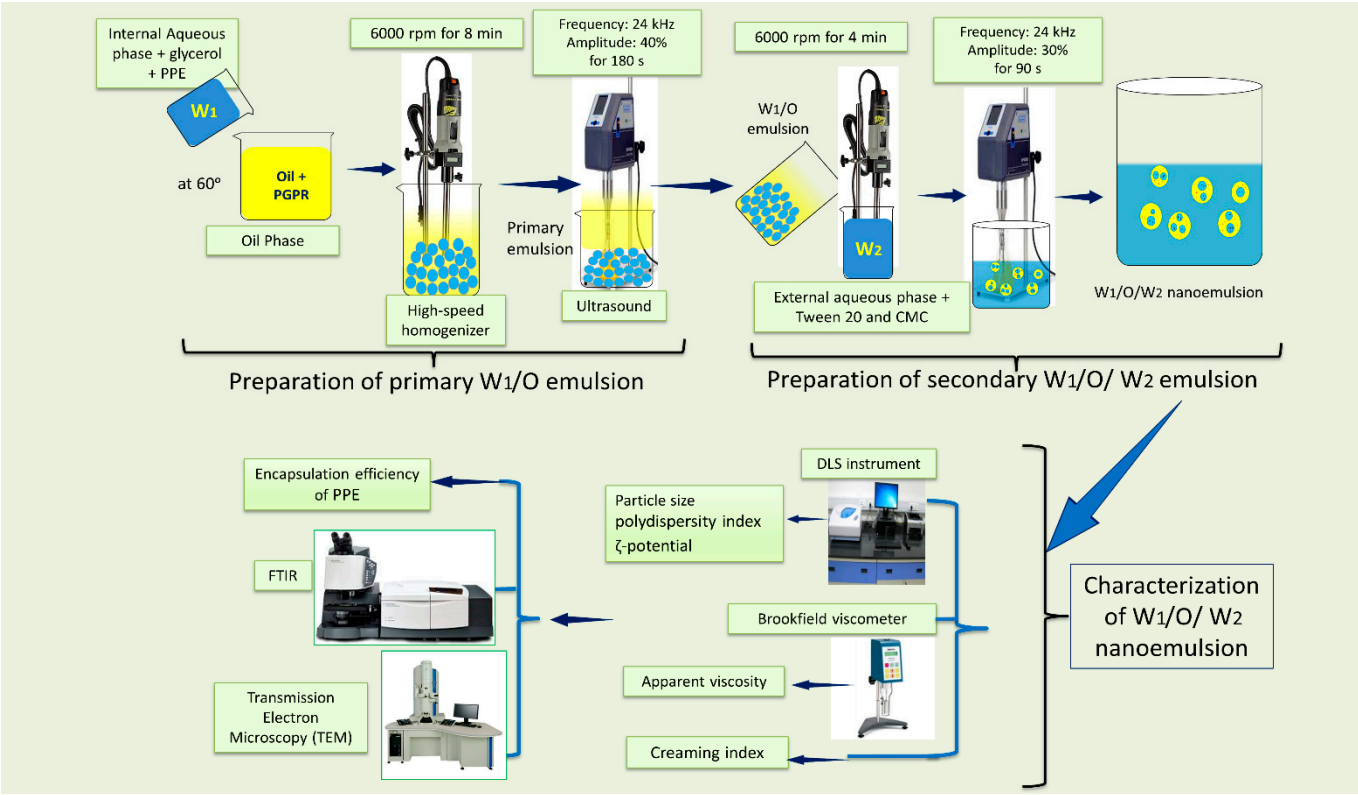

Figure 1. Shows the preparation and characterization of $W_1/O/W_2$ nanoemulsions.

2.5.2. Viscosity Measurement

Brookfield viscometer (Brookfield, WI, USA, DV-II Brookfield) was used to estimate the viscosity of double emulsions at controlled room temperature (25 °C) and 250 rpm with spindle No. 21. The viscosity value was read and reported in terms of centipoises (cp).

2.5.3. Creaming Index (CI)

Creaming of double emulsions was monitored through the storage at 4 °C by measuring the total emulsion height (EH) and the serum layer height (SH). The following formula was used to determine the creaming index (CI) [34]:

$$\mathrm{CI}(\%) = 100 \times \frac{\mathrm{SH}}{\mathrm{EH}} \quad (1)$$

Creaming of the $W_1/O/W_2$ emulsion led toseparating the aqueous phase at the bottom and the oil phase at the top, which was seen easily by the naked eye.

2.5.4. FTIR

FTIR of spectra of corn oil, PPE, CMC, double emulsion contained PPE in W_1 (PPE-DE), and double emulsion contained PPE in W_1 with CMC in W_2 (CMC/PPE-DE) were recorded using the Agilent Cary 630 FTIR-spectroscopy (Santa Clara, CA, USA). Samples were mixed with KBr powder, then pressed into a disk, and scanned in the frequency range of 650–4000 cm^{-1} at ambient temperature.

2.5.5. Measurement of EE

The EE (%) of double $W_1/O/W_2$ emulsions loaded with PPE was determined as the total phenolic content (PC) remaining in the primaryemulsions (W_1/O) during the emulsification process. The remaining PC content was determined according to Velderrain-Rodriguez et al. [29] with slight changes. Firstly, double $W_1/O/W_2$ emulsion samples were centrifuged (13,600× g/15 min/4 °C). Then, a syringe needle was used to gently

remove the bottom aqueous layer, which was then filtered through a 0.45 mm syringe filter to collect just water and exclude oil droplets. Thereafter, the recovered PC was measured bythe Folin–Ciocalteu method [35]. The results (mg GAE/g emulsion) were determined from a standard calibration curve (Figure 2). The equation below was used to calculate the EE (%) of emulsions:

$$EE(\%) = \left[1 - \frac{TP_S - TP_C}{TPP_{PE}}\right] \times 100 \quad (2)$$

where TPs: Total GAE found in PPE-DE and CMC/PPE-DE after centrifugation; TP_C: GAE value in the Blank-DE after centrifugation; TPP_{PE}: Total amount of GAE in the PPEdissolved inW_1 phase solution.

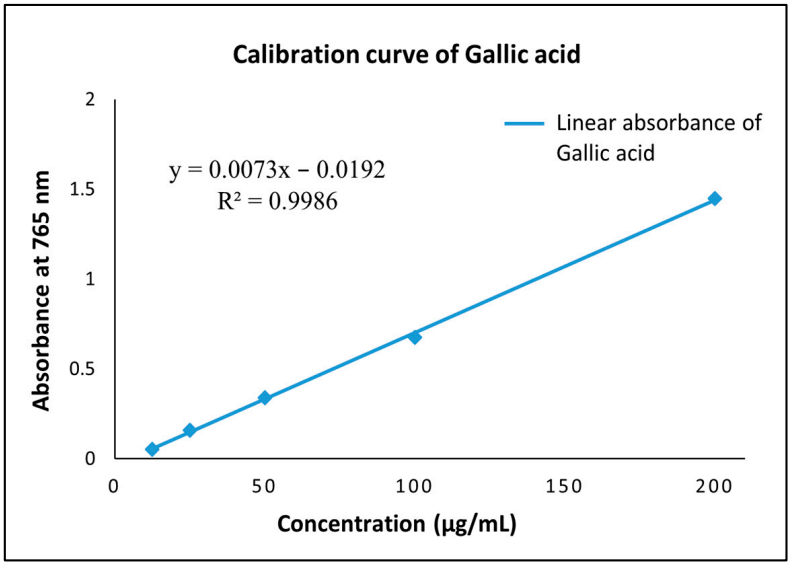

Figure 2. Gallic acid standard calibration curve.

2.5.6. TEM

The morphology of double $W_1/O/W_2$ emulsions was observed by TEM (JEOL GEM-1010, Tokyo, Japan) operated at 80 kV. The $W_1/O/W_2$ emulsions were diluted 10 times with distilled water, and the diluted emulsion was put onto carbon-coated copper grids (CCG) and retained for 1 min at ambient conditions. Then, Whatman filter paper was used to absorb the excess sample. The grid was stained with 2% uranyl acetate as a negative staining agent, and was left to dry before the TEM images were captured.

2.6. Statistical Analysis

Results are shown as mean ± SD. Statistical analyses were performed during one-way analysis of variance (ANOVA) using SPSS software (version 26); $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Droplet Size Distribution and ζ Potential

The particle size Intensity-Gaussian distributions and its related indices of the $W_1/O/W_2$ nanoemulsions loading with/without PPE are shown in Figure 3 and Table 1. The results revealed that emulsions containing phenolic compounds (PPE-DE and CMC/PPE-DE) have a lower particle size in comparison with Blank-DE. PPE-DE achieved the smallest size (259.2 nm); this decrease is mostly because of the surface activity of phenolic compounds, which lowered the interfacial tension at the oil/water interface [36,37].

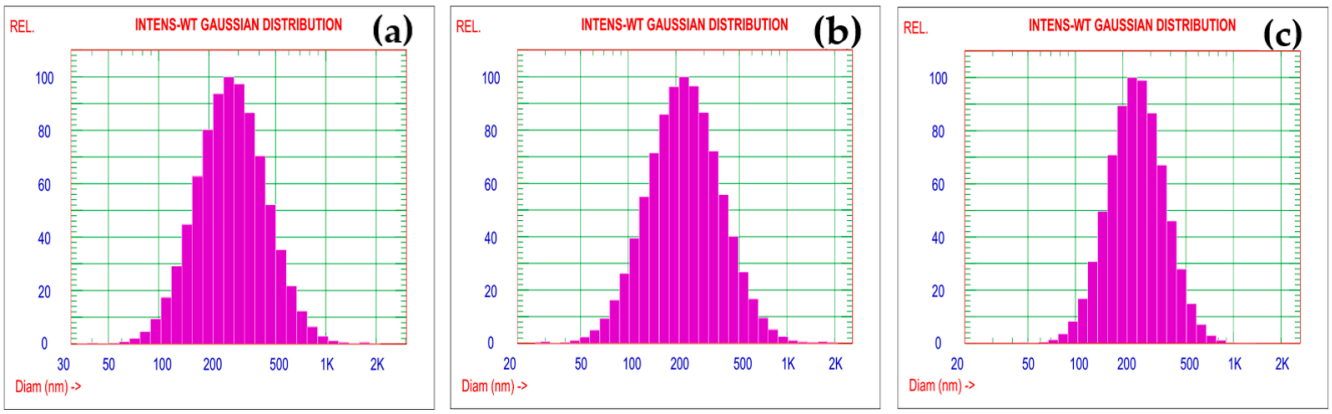

Figure 3. The particle size distribution of (**a**) Blank $W_1/O/W_2$ emulsion (Blank-DE); (**b**) DE containing pomegranate peel extract in the internal (W_1) phase (PPE-DE), and (**c**) DE containing pomegranate peel extract in the internal (W_1) phase with carboxymethyl cellulose in the external (W_2) phase (CMC/PPE-DE).

Table 1. Particle size and polydispersity index for different double nanoemulsions.

Sample	Mean Diameter (nm)	Standard Deviation (nm)	Chai Square	Variation PDI
Blank-DE	304.1	145.1	12.157	0.228
PPE-DE	259.2	137.4	1.091	0.281
CMC+PPE-DE	264.5	108.7	4.793	0.169

The PDI indicates the level of dispersion homogeneity, which ranged from 0 to 1. If this value is close to 0, it means that the dispersion particles are homogeneous in their size. If the PDI value > 0.5, it means the presence of non-uniform sized particles [38]. In our study, all the $W_1/O/W_2$ emulsions exhibited monodisperse (homogeneous) particle size distribution with PDIs less than 0.3, thus demonstrating high kinetic stability. The formulation prepared with CMC presented the lowest PDI value (0.169). Commonly, it is presumed that the smaller the droplet size, the more stable emulsion's stability, especially against creaming and sedimentation phenomena [39]. It seems that "average globule diameter and size distribution in the multiple emulsion might be influenced by factors such as nature and concentration of surfactants, aqueous and oil phase viscosity, oil phase composition, and production formulation conditions". According to Silva et al. [40], previous factors make the comparison among studies complicated.

Zeta potential has a significant role in the physical stability of the emulsions [41]. Notably, the higher value of zeta potential (positive or negative) indicates that the emulsions are more stable [42]. Figure 4 and Table 2 show the average value of zeta potential and its related indices of the $W_1/O/W_2$ nanoemulsions.

Table 2. Zeta potential measurements for different double nanoemulsions.

Sample	Avg. Zeta Potential (mV)	Cell Current (mA)	Avg. Mobility (M. U.)	Frequency Shift (Hz)
Blank-DE	−19.41	0.09	−1.36	−2.42
PPE-DE	−45.81	0.06	−3.20	−6.36
CMC/PPE-DE	−25.30	0.13	−6.36	−2.85

Zeta potential values were within a range of −19.41 to −45.81 mV, and the greatest potentials were obtained in the emulsion belonging to the PPE-DE sample, in which the smallest particle size was also achieved, hence exhibiting higher stability than other samples.

Generally, when the absolute value of zeta potential is more than 25 mV, emulsions are usually considered stable [43].

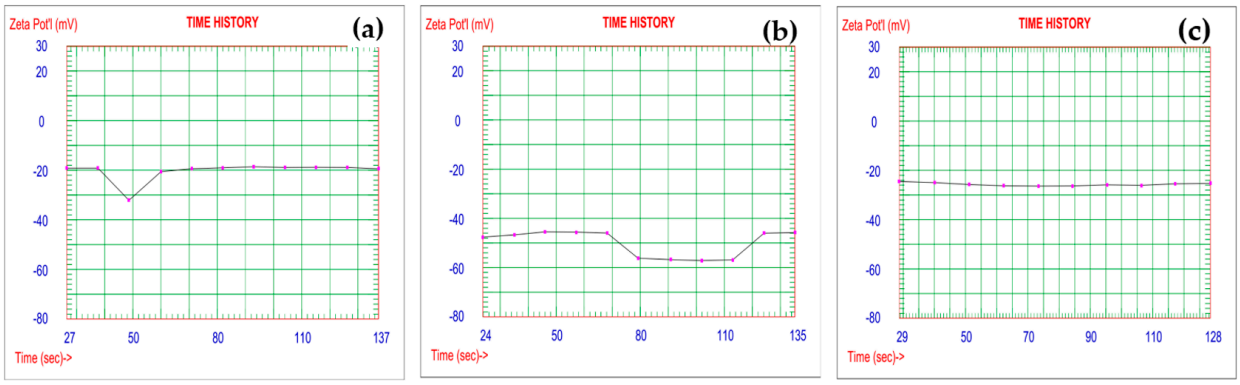

Figure 4. Zeta potential measurements of (**a**) Blank $W_1/O/W_2$ emulsion (Blank-DE); (**b**) $W_1/O/W_2$ emulsions containing pomegranate peel extract in the internal (W_1) phase (PPE-DE), and (**c**) $W_1/O/W_2$ emulsions containing pomegranate peel extract in the internal (W_1) phase with carboxymethyl cellulose in the external (W_2) phase (CMC/PPE-DE).

3.2. Viscosity and Creaming Index

Table 3 depicts the viscosity and CI of the $W_1/O/W_2$ nanoemulsions. The viscosity of Blank-DE exhibited the lowest value (3.4 cP), while it was significantly increased with the addition of phenolic-rich extract in the inner phase. This observation could be due to the interaction between the phenolic compounds in PPE and Tween 20 which are absorbed at the oil–water interface [29]. These findings are consistent with Velderrain-Rodríguez et al. [29], who reported that the $W_1/O/W_2$ emulsions containing mango peel phenolic extract had higher apparent viscosity as compared to control emulsion, and the $W_1/O/W_2$ emulsions made with Tween 20 or lecithin had the highest values of apparent viscosity.

Table 3. Shows the viscosity for different double nanoemulsions.

Sample	Viscosity (cP)	Creaming Index (% CI)		Thermodynamical Stability
		20 Days	30 Days	
Blank-DE	3.4 ± 0.07^{b}	51.87 ± 0.98^{a}	57.22 ± 1.18^{a}	Instable
PPE-DE	3.6 ± 0.10^{a}	1.55 ± 0.11^{b}	1.55 ± 0.11^{b}	Stable
CMC/PPE-DE	3.7 ± 0.12^{a}	0.52 ± 0.06^{b}	0.52 ± 0.06^{b}	Stable

Note: Values ± SD. Letters (a, b) mean significant differences in the same column.

In our study, the emulsion supplemented with 0.5% CMC in the outer phase was observed to have the highest viscosity. Several previous studies indeed reported that the addition of CMC or other biopolymers in double emulsions induced a considerable increase of viscosity [25,44].

On the other hand, the creaming index expresses the emulsion's stability in relation to the separation process of the phases of an emulsion. "It is influenced by many factors such as the difference in densities between the aqueous and oil phases, the viscosity of the continuous phase, the interactions between the droplets of the discontinuous phase, etc." [45].

At the beginning, fresh $W_1/O/W_2$ nanoemulsions showed no signs of phase separation irrespective of the formulation. Posteriorly, creaming of Blank-DE increased significantly with the storage time and reached out to 51.87 and 57.22% at 20 and 30 days, respectively (Figure 5). In contrast, we observed an extreme little separation at the top in PPE-DE and CMC/PPE-DE formulations after 20 days of storage and it remained unchanged after 30 days; in other words, emulsion stability evolved in the order, CMC/PPE-DE > PPE-DE > Blank-DE, as shown in Table 3, which agreed with the viscous character increment dis-

cussed previously in Section 3.2. This superior stability of emulsion containing phenolic compounds compared to blank emulsion might be due to these phenolic compounds working as emulsifiers and forming stable emulsions. Finally, our findings were in agreement with that reported by Ye et al. [46], who noted that integration of polysaccharides into the continuous aqueous phase (W_2) at a concentration of >0.1% avoids creaming throughthe storage period.

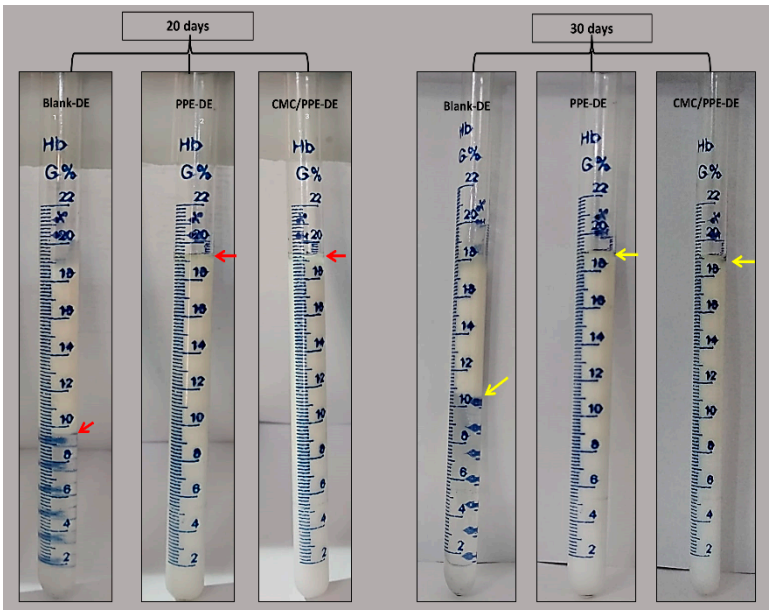

Figure 5. Digital photographs of Blank double emulsion (Blank-DE); DE containing pomegranate peel extract in the inner (W_1) phase (PPE-DE), and DE containing pomegranate peel extract in the inner(W_1) phase with CMC in the external (W_2) phase (CMC/PPE-DE) at 20, 30 days.

3.3. FTIR Analysis

FTIR spectra of raw materials (CMC, PPE, and corn oil) and the prepared emulsions (PPE-DE and CMC/PPE-DE) are presented in Figure 6.

Figure 6a is the FTIR spectra of the corn oil; the large peak at 1744 cm^{-1} corresponding to absorption by carbonyl double bonds (C=O stretching) of the free fatty acids (oleic and linoleic acids) found in corn oil [32]. Notably, the band at 3008 cm^{-1} is due to the =C–H stretching vibration. The spectrum had strong band absorptions in the region of 3000–2800 cm^{-1} caused by C–H stretching vibrations. Methylene (–CH2–) and methyl (–CH3) stretching vibrations occur at frequencies of 2922 and 2851 cm^{-1}, respectively [47]. Figure 6b represents the FTIR spectrum of pomegranate peel extract. Five bands appeared at 3321, 2355, 2124, 2001, and 1636 cm^{-1} are assigned to active groups. Mainly, the major peaks were obtained at 3321 and 1636 cm^{-1}. The 3321 cm^{-1} peak corresponded to -NH, and the -OH groups and the 1636 cm^{-1} peak were attributed to stretching vibration of C=C of the aromatic compounds [48]. However, the weak band at 2355 cm^{-1} indicates carbonyl-specific absorption.

Figure 6c shows the FTIR spectrum of CMC, the peak at 1408 cm^{-1} was due to symmetrical stretching vibrations of COO- groups [49].The band at 2892 cm^{-1} corresponded to carbon-hydrogen bond (C-H) stretching [50]. Figures 5e and 6d are the FTIR spectrum of PPE-DE and CMC/PPE-DE, respectively. Bands at 3321, 2355, and 1636 cm^{-1} in Figure 6d, and peaks at 3354, 2355, and 1636 cm^{-1} in Figure 6e indicate that the spectrum of pomegranate peel extract is present in the $W_1/O/W_2$ nanoemulsions, and other bands in Figure 6d,e are similar to peaks corresponding to the triglycerides functional groups present in the corn oil. Thus, it can be concluded from the FTIR study that the components were present in the $W_1/O/W_2$ emulsion samples; however, the observed shift in wavenumbers indicates a strong interaction between the emulsion components.

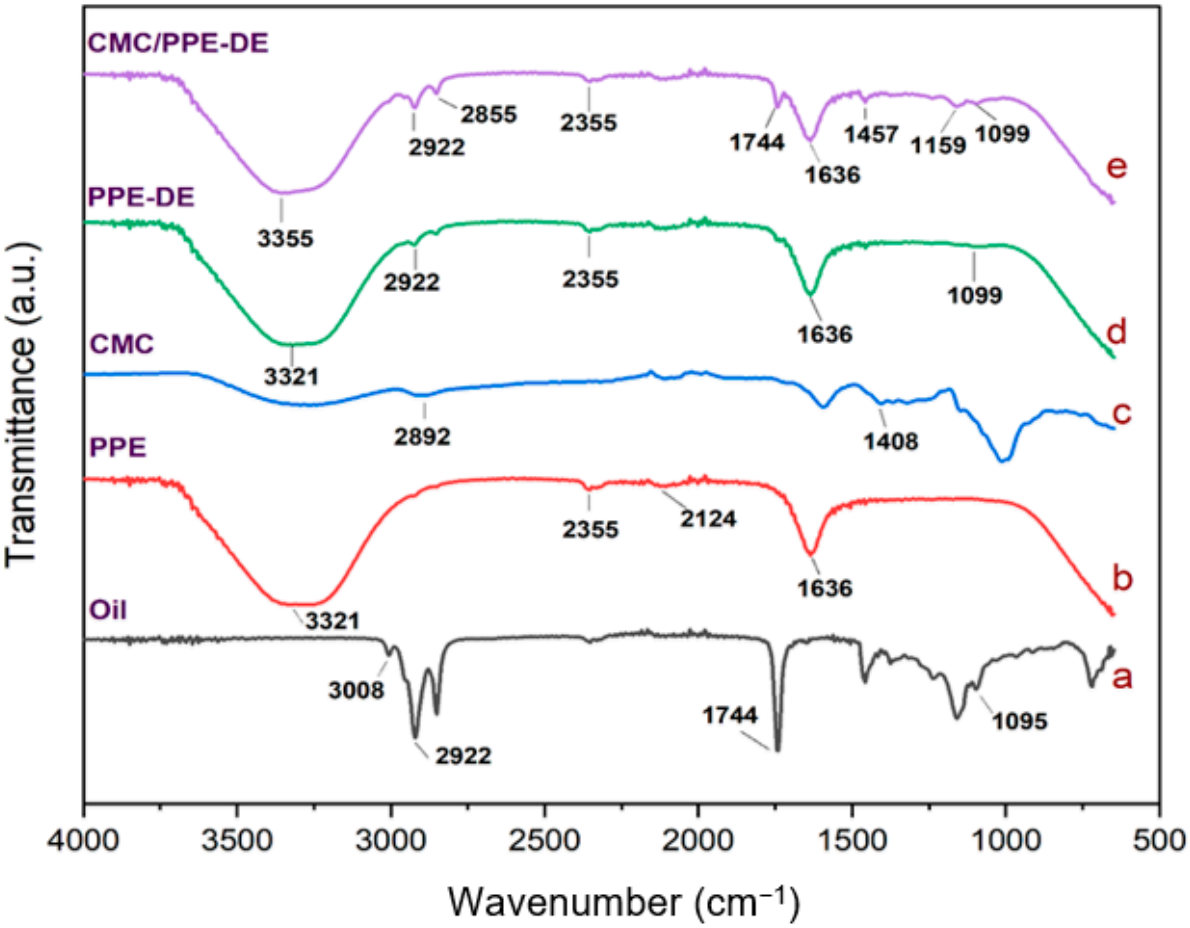

Figure 6. FTIR spectra of (**a**) Corn oil; (**b**) pomegranate peel extract (PPE); (**c**) carboxymethyl cellulose; (**d**) DE containing pomegranate peel extract in the internal (W_1) phase (PPE-DE), and (**e**) DEcontaining pomegranate peel extract in the internal (W_1) phase with CMC in the external (W_2) phase (CMC/PPE-DE) scanned in mid-infrared region (wavenumbers 4000–500 cm^{-1}).

3.4. Encapsulation Efficiency

The Encapsulation Efficiency (EE) is a significant parameter determining the effectiveness of $W_1/O/W_2$ emulsion for retaining PPE in the internal aqueous phase. Furthermore, it has been suggested that a higher value of encapsulation efficiency is a direct consequence of the good stability of double emulsions [51]. Here, the initial EE of double $W_1/O/W_2$ emulsions was 96.1% for the PPE-DE, whereas it was 95.4% for those containing CMC (Figure 7). This finding may be attributed to using the lipophilic emulsifier PGPR, which verifies a high-water EE [52] attributable to a steric stabilization of the interfacial layer [53]. Many researchers have reported similar results; Velderrain-Rodríguez et al. [29] reported that the double emulsion formulated using PGPR and Tween 20 as the first and second surfactants, respectively, had the higher encapsulation efficiency (98.65 ± 1.14%) of mango peel extract. Likewise, Aditya et al. [26] fabricated $W_1/O/W_2$ emulsions loaded with catechin inside the internal aqueous phase using PGPR as a lipophilic emulsifier and found that the encapsulation efficiency of catechin was 97 ± 0.3%. Wang et al. [54] found good encapsulation efficiency (over > 95%) between PGPR and Tween 80, showed EE > 95%.

After 30 days of storage, EE slightly decreased by 6% for PPE-DE and 4.4% for CMC/PPE-DE; the higher stability in the latter may be due to the synergistic effect that occurred when Tween 20 and CMC were used together in W_2 phase. Mohammadi et al. [30] observed that using a hydrophilic emulsifier of WPC and pectin in the outer aqueous phase of W/O/W emulsion has led to increasing the EE and reducing the release of PC through storage. Matos et al. [44] also encapsulated trans-resveratrol in the W/O/W emulsion

with a hydrophobic emulsifier (PGPR). He found that the recovery yield values were 97.84 ± 2.96% using Tween 20 and 95.14 ± 3.37% using Tween 80. The authors also suggested that the system with sodium carboxymethyl cellulose leads to better encapsulation efficiency values.

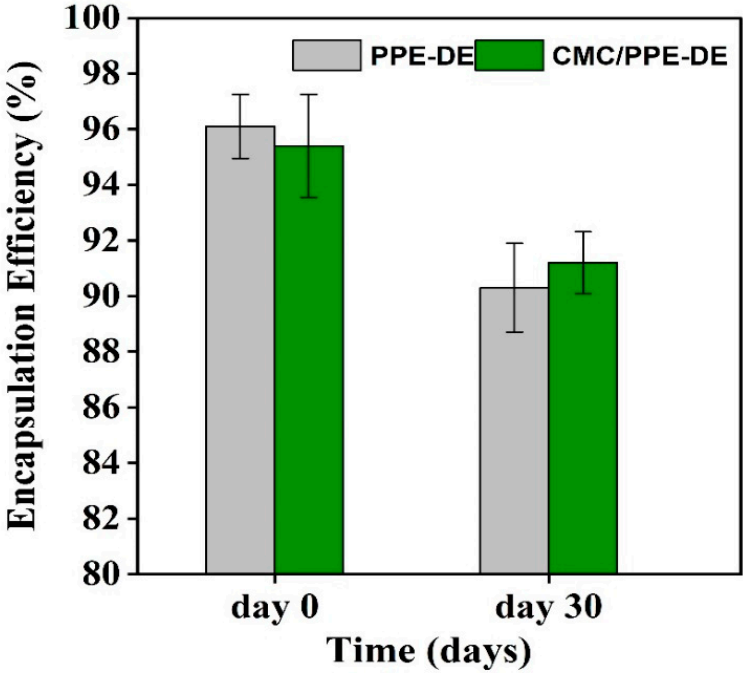

Figure 7. Encapsulation efficiency of DE containing pomegranate peel extract in the inner (W_1) phase (PPE-DE), and DE containing pomegranate peel extract in the inner (W_1) phase with CMC in the external (W_2) phase (CMC/PPE-DE).

3.5. Morphology of $W_1/O/W_2$ Nanoemulsions

TEM is an extremely useful technique that provides a deeper understanding of the microstructure characterization of colloidal systems, such as $W_1/O/W_2$ emulsions. As shown in Figure 8, the TEM images of the three formulations of $W_1/O/W_2$ nanoemulsions: Blank-DE, EPP-DE, and CMC/EPP-DE show that the W_1/O emulsion droplets were uniformly distributed in the continuous phase (W_2) as can be seen in the obtained images (Figure 8a–c). This homogeneous distribution indicates the successful preparation of the $W_1/O/W_2$ nanoemulsions. However, the reverse micelle structures inside the oil droplets were too small to be clearly visualized with this technique [55]. It was also notedthat the particle size distribution for all emulsions was narrow, which was consistent with the previous PDI values obtained by DLS (Section 3.1). Furthermore, the W_1/O droplets in all formulations appeared spherical in their shape. The TEM images showed the absorption of the hydrophilic emulsifiers used at the O–W_2 interface, resulting in the formation of a compact layer on the surfaces of the spherical oil droplets, thereby enhancing the stability of emulsions. Besides, the images revealed that the three emulsions had individual particles (Figure 8a–c), indicating that there was no sign of flocculation in these emulsions.

As anticipated, the particle sizes determined with TEM (Figure 8a–c) were smaller than those obtained by the DLS instrument (Figure 3, Table 1). This difference is because of the measurements by TEM require the emulsion to be in a dry state by air-drying. In contrast, DLS measures the hydrodynamics of the dispersed particles [56].

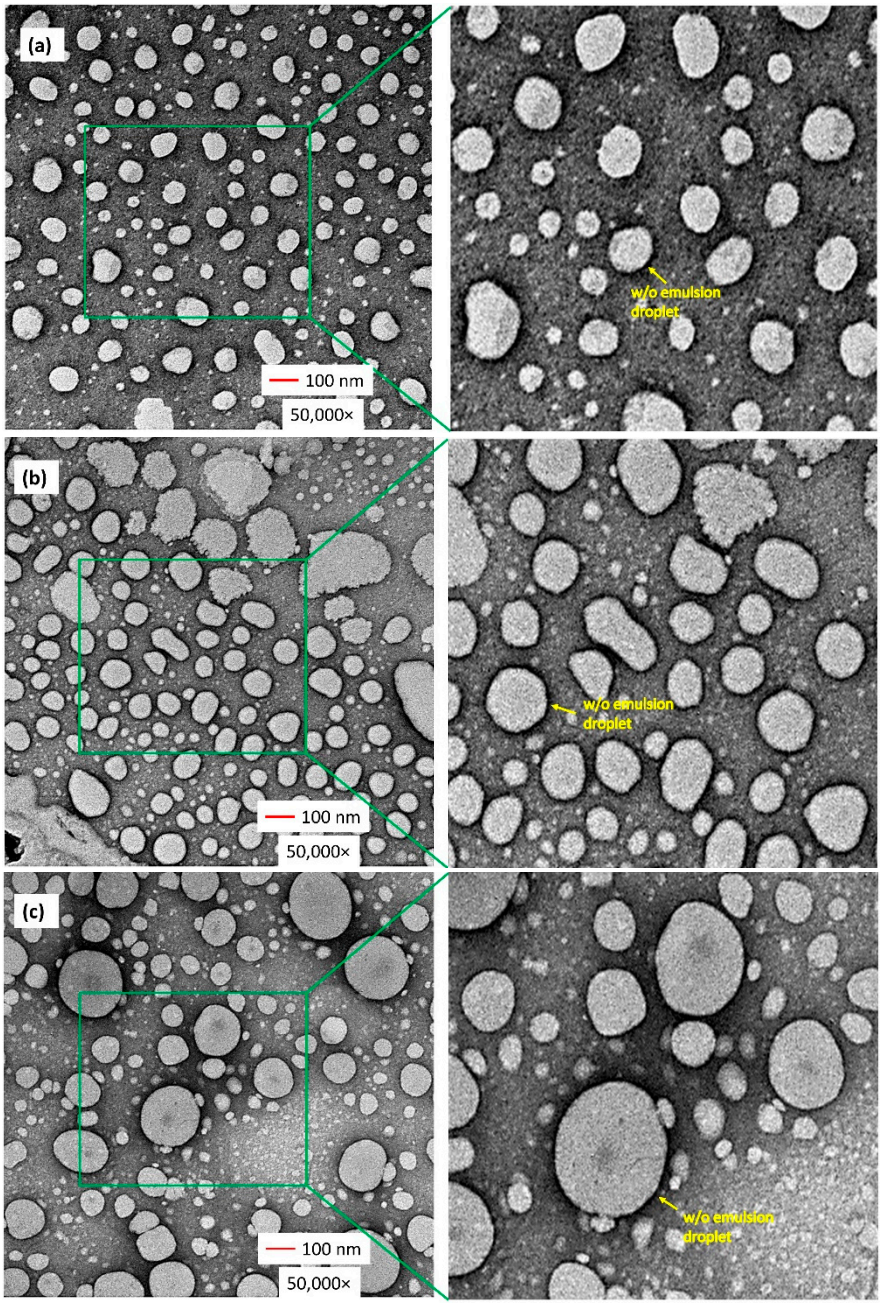

Figure 8. TEM Image of (**a**) Blank (Blank-DE); (**b**) DE containing PPE in the internal (W_1) phase (PPE-DE), and (**c**) DE containing PPE in the internal (W_1) phase with CMC in the external (W_2) phase (CMC/PPE-DE).

4. Conclusions

To sum up, a food-grade $W_1/O/W_2$ emulsions system was effectively fabricated to encapsulate the natural bioactive compounds extracted from pomegranate peel through a two-step emulsification method. The phenolic-rich inner aqueous phase (W_1) was emulsified in corn oil with PGPR as a hydrophobic emulsifier, while the oil droplets of the double $W_1/O/W_2$ emulsion were stabilized with Tween 20 as a hydrophilic emulsifier. The formulated emulsions loaded with PPE, both with or without CMC, were more stable with smaller particle size and PDI than the blank emulsion. Moreover, these emulsions exhibited excellent initial encapsulation efficiency and phenolics retention during storage. Nevertheless, the emulsion with CMC showed the highest stability compared to the emulsion without CMC. The FTIR spectra showed that the PPE and the other ingredients were

presented in the emulsion, while the TEM images showed that the prepared emulsions had good morphology.

Thus, our work suggests that the $W_1/O/W_2$ emulsion system can be used as an applicable and effective method to encapsulate phenolic-rich extract from pomegranate peel. Therefore, the release and bioavailability characteristics of double emulsions loaded with phenolic-rich extract from pomegranate peel are recommended for future work.

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