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Research Article

Application of CMC as Thickener on Nanoemulsions Based on Olive Oil: Physical Properties and Stability

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Carboxymethyl cellulose (CMC) is a hydrocolloid with surface activity that could act as emulsifiers in oil-in-water emulsions; however the principal role is that it acts as structuring, thickening, or gelling agent in the aqueous phase. This study aims to evaluate the application of CMC as thickener into nanoemulsions based on olive oil and their influence on particle characteristics, flow behavior, and color. Four nanoemulsions with different oil (5% and 15% w/w olive oil) and CMC (0.5% and 0.75% w/w) concentration and two control samples without CMC added were prepared using Tween 80 as emulsifier. All physical properties studied on nanoemulsions were depending on both oil and CMC concentration. In general, z-average particle size varied among 107–121 nm and those samples with 5% oil and CMC were the most polydisperse. The addition of CMC increased anionic charge of nanoemulsions obtaining zeta potential values among −41 and −55 mV. The oil concentration increased both consistency and pseudoplasticity of samples, although samples were more stable to gravitational separation at the highest CMC concentration. Color of nanoemulsions was affected principally by the oil concentration. Finally, the results showed that CMC could be applied in nanoemulsions as thickener increasing their physical stability although modifying their physical properties.

1. Introduction

There has been a growing interest in the use of lipid-based delivery systems, particularly useful for encapsulating and releasing lipophilic bioactive components, including liposomes, conventional emulsions, microemulsions, and nanoemulsions [1–4]. Oil-in-water (O/W) nanoemulsions are a specific type of colloidal dispersion characterizing by very small oil droplet sizes dispersed within an aqueous phase, with each droplet being coated by a protective coating of surface-active molecules and having a radius <100 nm [5–7]. The reduction in the droplet size of O/W emulsions may be beneficial over other types of delivery systems for certain food applications, such as more stability to droplet aggregation and gravitational separation, bulk viscosity, high optical clarity, and its ability to increase the bioavailability of encapsulated active ingredients during digestion [8–11].

Nanoemulsions can be produced from food-grade ingredients using different processing operations, such as mixing, shearing, and homogenization [7]. However, the physicochemical properties of the oil-water interface and homogenization mechanism have a strong impact on physical stability of emulsions. Most polysaccharides behave as emulsion stabilizers by forming an extended network in the continuous phase, which thus becomes highly viscous [11, 12]. Therefore, they can modify the rheological behavior of continuous phase and have an important effect on physical stability of emulsions. These hydrocolloids should be selected according their ability to tailor the desirable physicochemical and sensory attributes such as appearance, texture, and flavor profile [13]. Among food hydrocolloids, cellulose derivatives have gained acceptance for pharmaceutical, cosmetic, food, and packaging uses. They are obtained by replacing the hydroxyl groups with either alkyl or hydroxy-alkyl groups in the cellulose chain.

Carboxymethyl cellulose (CMC), also known as cellulose gum, is an anionic linear polymer, long chain and water soluble, which is manufactured by chemically attaching carboxymethyl groups to the backbone through reacting alkali cellulose with sodium monochloroacetate [14, 15]. Due to its ionic nature, viscosity of CMC dispersions is sensitive to pH and ionic strength, as well as to the presence of other types of electrically charged molecules [14, 16]. In food industry CMC may act as a thickener, emulsion stabilizer, moisture binder, and suspending and improving texture of a wide variety of food products [17] and has the advantage that it is physiologically inert and noncaloric [18]. Besides CMC is thoroughly used because it is odorless and tasteless and forms clear solution without cloudiness or opacity and is commonly used in foods and beverages to prevent gravitational separation of suspended particles and to create desirable textural attributes and mouthfeel [14–16].

In the last time, CMC has been used as an alternative thickener to starch in semisolid dairy products [19–22] due to its technological, sensory, and nutritional advantages. However, less information exists regarding how the CMC acts as thickening agent on food emulsions. In recent studies, the authors reported that CMC macromolecules exhibited a dominating effect on emulsion flow behavior, although the presence and concentration of fat droplets also played an important role [21].

On the other hand, it is known that the intake of oils with healthy features as part of the daily diet helps in preventing cardiovascular disease, hypertension, and some cancers [23, 24]. Olive oil is a natural source of several bioactive compounds as unsaponifiable and soluble fraction, which includes phenolic compounds [25], and has very good organoleptic properties, which could be used in the formulation of foods with healthy features. However, the type of oil not only affects sensory and nutritional properties, but also rheology and stability of emulsions. Each type of oil has its own particular challenges and depends on its physicochemical properties such as solubility, the length-chain of fatty acid, and chemical stability [26, 27]. Thus, it is important to understand the major components influencing the formulation of food emulsions and their effects on physical properties to obtain delivery systems based on nanoemulsions with long-term stability. In this context, the main objective of this work was to evaluate the effect of CMC concentration on physical properties and stability of nanoemulsions with different olive oil content in order to apply CMC as thickener on nanoemulsions.

2. Materials and Methods

2.1. Composition and Preparation of Oil/Water (O/W) Nanoemulsions. Oil-in-water (O/W) nanoemulsions were prepared with extra virgin olive oil (Casta de Peteroa, Terramater S.A., Chile) as dispersed phase, carboxymethyl cellulose (CMC) (CEKOL 30000, Quimatic S.A., Chile) as thickener, Tween 80 (Sigma-Aldrich S.A., France) as emulsifier/surfactant, and purified water from an inverse osmosis system (Vigaflow S.A., Chile). Different formulations were prepared at two oil concentrations (5 and 15% w/w oil) and two CMC concentrations (0.5 and 0.75% w/w CMC). For each oil concentration, a control sample without thickener was prepared. The surfactant-to-oil ratio used was 6 : 5, which was previously determined by the optimization of sonication-processing conditions (surfactant-to-oil ratio and sonication time) by Surface Response Methodology (data not shown).

The aqueous phase was prepared dispersing Tween 80 in the purified water using magnetic stirrer (Arex, Velp Scientifica, Italy) at 200 rpm for 30 min at room temperature. Then, oil was added slowly to the aqueous phase and the mixture was stirred using a rotor-stator homogenizer (Wiggen Hauser D130, Germany) at 21200 or 16800 rpm (for samples with 5% or 15% oil, resp.) for 10 min in a water bath at $5 \pm 1^{\circ}$C to avoid warming of samples. In order to obtain nanoemulsions, the coarse emulsions were then homogenized using an ultrasonic processor (VCX500, SONICS & Materials, USA) with a 13 mm (diameter) stainless steel ultrasound probe. Sonication was performed at 80% amplitude, in a pulsed mode of 15 s and 5 s of rest, and at a frequency of 20 Hz, for 21 and 16 min for samples with 5 and 15% oil, respectively.

After that, CMC was added to the O/W nanoemulsions and mixed using a propeller stirrer at 1500 rpm (BS, Velp Scientifica, Italy) at room temperature until their complete dispersion (approximately 40 min). Finally, all samples were transferred to a closed flask and stored under refrigeration ($5 \pm 1^{\circ}$C for 24 h) prior to measurements. At least two batches of each nanoemulsion were prepared.

2.2. Particle Characterization

2.2.1. Particle Size. The droplet size and its distribution for each nanoemulsion were measured by Dynamic Light Scattering (DLS) using a Zetasizer NanoS90 (Malvern Instruments Ltd., UK). A refractive index value of 1.47 was used for the disperse phase (olive oil) and of 1.33 for the continuous phase (water), which was determined by a refractometer (RA-130, Kyoto Electronics, Japan). The particle size of samples was described by the zeta-average particle size (PS) and the size distribution was described by the polydispersity index (PdI) and the size distribution graph. Measurements were made in triplicate for each sample.

2.2.2. Particle Electrical Charge. Based on DLVO (Deyaguin-Landau-Verwey-Overbeek) theory, the emulsion stability is affected by the magnitude of repulsive and attractive forces between emulsion droplets [28]. To evaluate this, zeta potential of each emulsion was determined using a particle electrophoresis instrument (Zetasizer Nano-ZS series, Malvern Instruments, UK). Particle charge data was collected over 30 continuous readings. All measurements were made in duplicate with fresh sample and the zeta potential measurements were reported as the mean of three separate measurements.

2.3. Flow Behavior. Nanoemulsions were measured in a rotational rheometer (RheolabQC, Anton Paar, Austria) using cylinder sensor CC27 for samples with CMC and DG42 for control samples. At least, two batches of each composition were measured in duplicate at a controlled temperature of 10 ± 1°C and after loading the samples, it was allowed to stand for 2 min to stabilize and reach the desired temperature.

Flow curve was obtained by recording shear stress values when shearing the samples at linearly increasing shear rates from 1 to 200 s^{-1} through 120 s and down in reverse sequence for the same time [21]. Experimental data from ascending flow curve were fitted to the Ostwald-de Waele model:

$$\sigma = K\dot{\gamma}^{n}, \tag{1}$$

where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index (Pa s^{n}), and n is the flow behavior index.

2.4. Stability. In order to determine the storage stability, the samples were analyzed using a vertical scan analyzer (Turbiscan MA 2000, Formulaction, France). All emulsions were poured in cylindrical glass tubes (total height ~60 mm) and stored for 21 days at 5 and 20°C. The selected temperatures are related to potential storage temperatures of these nanoemulsions. The physical phenomena of destabilization were evaluated through the backscattering (BS) profiles as a function of the sample height into the cylindrical glass.

During storage period, each emulsion showed a cream layer at the top and serum layer at the bottom of cylinders. The extent of creaming was characterized by the creaming index H, since the higher creaming index is representative of the emulsion instability. Normally, the creaming index should start at zero and increase during storage until a fairly constant value, which is reached when all the droplets are packed tightly into the cream layer [16]. This parameter was calculated according to (2) described by Petrovic et al. [29]:

$$\text{Creaming index (\%)} = \left(\frac{H_S}{H_E}\right) \times 100, \tag{2}$$

where H_E is total height of the emulsion (mm) and H_S is the height of cream layer (mm) which were measured as a function of time. All measurements were performed in triplicate for each emulsion.

2.5. Color. Digital images from each nanoemulsion (15 mL samples in a Petri plate of 53 mm diameter and 10 mm height) were captured on a white background through a computer vision system setup, consisting of a black box with four natural daylight (D65) tubes of 18 W (Philips) and a digital camera (Canon Powershot G3 14 MP, Japan) at a distance of 22.5 cm from sample. The camera lens angle and light were at 45° according to Pedreschi et al. [30]. The camera was calibrated using 30 color charts with a colorimeter (CR-410, Konica Minolta, USA). All images were acquired at the same conditions and the camera was remotely controlled by EOS Utility software (Canon, USA). Images were analyzed extracting color values in RGB (red, green, and blue) space using Adobe Photoshop v7.0 program (Adobe Systems Incorporated, USA) and then converted to CIE $L^{*}a^{*}b^{*}$ space, where L^{*} is black-white component (lightness), a^{*} is red-green component, and b^{*} is yellow-blue component. The variation of color between day 0 and different storage times (days: 7, 14, and 21) at 5°C was calculated using CIE ΔE_{2000} equation [31]. A color difference (ΔE_{2000}) value higher than 1.5 indicates a subjective assessment of "noticeable" perception of color differences [32].

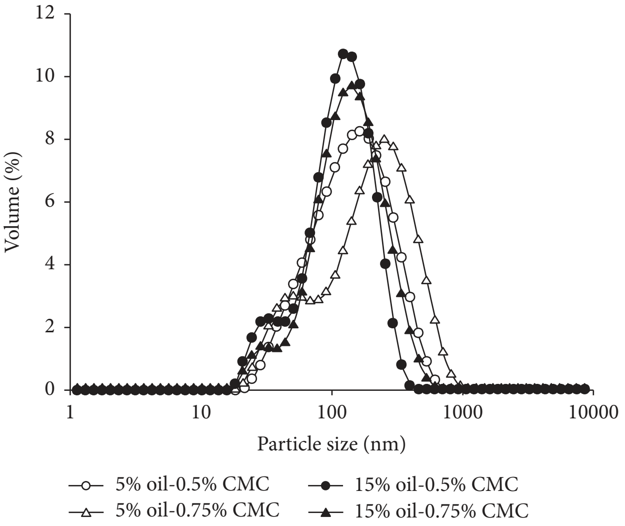


Figure 1: Particle size distribution of O/W nanoemulsions with different oil (5%: empty symbols and 15%: filled symbols) and CMC concentration (0.5%: circle and 0.75%: triangle).

2.6. Statistical Analysis. A two-way ANOVA (oil concentration and CMC concentration) with interaction between factors was applied to the data. Tukey's test ($\alpha = 0.05$) was used to calculate the minimum significant difference. All calculations were carried out with XLSTAT Pro software 2015 (Addinsoft, Paris, France).

3. Results

3.1. Particle Characterization. Figure 1 shows the size distribution graphs, which indicate the droplet distribution of different nanoemulsions with CMC based on volume percentage of droplets as a function of zeta-average size. All samples exhibited monomodal particle distribution, but the width of the distributions decreased with increasing oil concentration. In general, the zeta-average particle size (PS) of different samples varied slightly among 106.7 and 121.3 nm according to the composition; however, sample polydispersity increased strongly with CMC concentration in nanoemulsions with 5% oil, but not on those with 15% oil. This difference in the effect of adding theoretically similar CMC concentration on polydispersity of nanoemulsions with different oil concentration can be due to the higher effective concentration of CMC in the aqueous phase of nanoemulsions with 15% oil. An increase in viscosity of the aqueous phase when CMC concentration increases can minimize droplet mobility, delaying collision frequency, and reducing droplet coalescence [21]. In the case of nanoemulsions with 5% oil, it was observed that CMC addition increased their polydispersity due probably

Table 1: Two-way ANOVA of particle size (PS), polydispersity index (PdI), and zeta potential (ZPot) for O/W nanoemulsions based on olive oil with different CMC concentrations. *F* and *p* values.

Parameters	Main effects				Interactions	
	A: oil concentration		*B*: CMC concentration		$A \times B$	
	F	*p*	*F*	*p*	*F*	*p*
PS (nm)	0.08	0.79	8.00	<0.01	1.72	0.21
PdI (—)	19.23	<0.01	5.34	0.02	3.61	0.04
ZPot (mV)	10.40	<0.01	938.48	<0.01	10.33	<0.01

Table 2: Mean values and significant differences of particle size (PS), polydispersity index (PdI), and zeta potential (ZPot) for O/W nanoemulsions based on olive oil with different CMC concentrations.

Oil concentration (% w/w)	CMC concentration (% w/w)	PS (nm)	PdI (—)	ZPot (mV)
5	0	106.65 ± 2.56^{b}	0.28 ± 0.02^{b}	-19.18 ± 0.79^{b}
	0.5	119.50 ± 5.49^{a}	0.40 ± 0.07^{a}	-45.55 ± 1.43^{d}
	0.75	121.33 ± 3.36^{a}	0.41 ± 0.09^{a}	-52.50 ± 0.91^{e}
15	0	112.96 ± 0.62^{ab}	0.26 ± 0.02^{b}	-14.05 ± 0.79^{a}
	0.5	118.45 ± 7.52^{ab}	0.27 ± 0.02^{b}	-41.43 ± 3.10^{c}
	0.75	117.90 ± 8.69^{ab}	0.28 ± 0.01^{b}	-54.76 ± 2.24^{e}

$^{a–e}$Means within a column with common superscripts did not differ significantly ($p > 0.05$).

to lower concentration of CMC into the aqueous phase, which it can not be enough to reduce droplet flocculation and coalescence. Nanoemulsions were measured after 24 h of storage; therefore the polydispersity of samples can be affected by the mechanisms of emulsion destabilization, as coalescence and flocculation. Besides, at very low concentration, the added hydrocolloids have a destabilizing effect on the emulsions, since the depletion flocculation induced by the nonadsorbing hydrocolloids causes enhanced serum separation of the emulsions [11].

ANOVA results showed that only CMC concentration affected significantly ($p < 0.05$) particle size (PS) of nanoemulsions (Table 1), but only significant differences ($p < 0.05$) on PS were found between the sample with 5% oil and 0.75% CMC and control sample with 5% oil (Table 2). Regarding polydispersity index (PdI), oil and CMC concentration as well as their interaction had a significant effect ($p < 0.05$) on PdI values (Table 1). The effect of CMC was different depending on oil concentration; in samples with 5% oil, the addition of CMC increased PdI values from 0.28 to 0.41, but in the 15% oil-nanoemulsions, PdI values almost did not vary (from 0.26 to 0.28) (Table 2). It can be due, as previously it was commented, to the increase of viscosity of continuous phase that reduces particle movement which delayed the phenomena of coalescence and flocculation.

Electrical charge of nanoemulsions, measured as zeta potential (ZPot), was affected significantly ($p < 0.05$) by oil and CMC concentration and by binary interaction of both factors (Table 1); however the factor that more influenced ZPot was CMC concentration (*F* value = 938.48). Control samples (without CMC added) showed ZPot values of −19.18 and −14.05 mV for samples with 5 and 15% oil, respectively. These results are in agreement with other studies where nanoemulsions were stabilized with the same nonionic surfactant (Tween 80) [33, 34]. Also, it has been reported that a nonionic emulsifier/surfactant can give a negative charge to oil droplets, due to preferential adsorption of hydroxyl ions from the aqueous phase or due to the presence of anionic impurities such as free fatty acids in the surfactant or oil phases [16].

When CMC was added into nanoemulsions, the magnitude of ZPot values increased significantly ($p < 0.05$) for both oil concentrations (Table 2). Samples with 0.5% CMC and different oil concentration showed ZPot values significantly different ($p < 0.05$), but when CMC was increased to 0.75% no differences were found between electrical charges of nanoemulsions with different oil content. Nevertheless, the strong negative ζ-potential observed in these nanoemulsions is due to the presence of CMC, which is an anionic hydrocolloid. The anionic groups in the polymer chain of CMC and its surface properties can stabilize the nanoemulsions, since they can be absorbed to the interfacial layer, but their stabilizing action depends on the possible interactions and competition between previously adsorbed species [11, 35].

Finally, it has been reported that emulsions with zeta potential more positive than +30 mV or more negative than −30 mV can be considered stable, since electrical charge of droplets is strong enough to assume that repulsive forces between droplets are predominant in the system [34, 36]. Thus, the nanoemulsions with CMC could be considered stable against the physical mechanisms of destabilization.

3.2. Flow Behavior. Figure 2 shows flow curves of CMC-based nanoemulsions with different oil concentration. All samples with CMC exhibited Non-Newtonian and shear

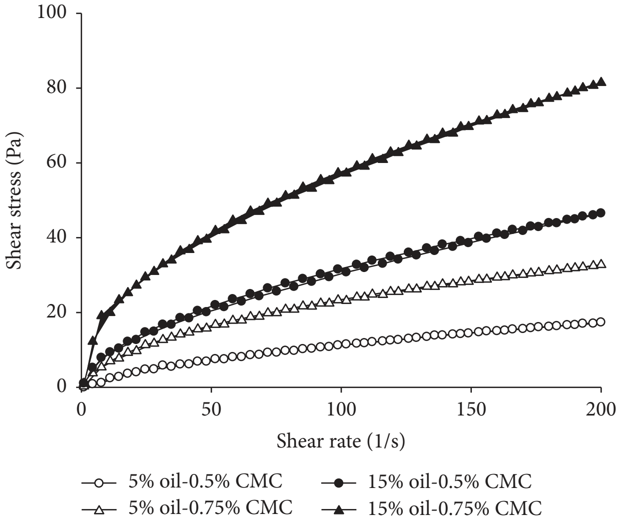


Figure 2: Flow curves of O/W nanoemulsions with different oil (5%: empty symbols and 15%: filled symbols) and CMC concentration (0.5%: circle and 0.75%: triangle).

Table 3: Mean values and significant differences of flow parameters (K: consistency index and n: flow behavior index) for O/W nanoemulsions based on olive oil with different CMC concentrations.

Oil concentration (% w/w)	CMC concentration (% w/w)	K (Pa s^n)	n (—)
5	0	0.001 ± 0.0001^{d}	1.01 ± 0.001^{a}
	0.5	0.53 ± 0.01^{c}	0.65 ± 0.01^{b}
	0.75	2.02 ± 0.05^{b}	0.53 ± 0.01^{c}
15	0	0.01 ± 0.001^{d}	1.01 ± 0.001^{a}
	0.5	2.21 ± 0.10^{b}	0.57 ± 0.01^{d}
	0.75	4.78 ± 0.26^{a}	0.49 ± 0.01^{e}

$^{a-e}$Means within a column with common superscripts did not differ significantly ($p > 0.05$).

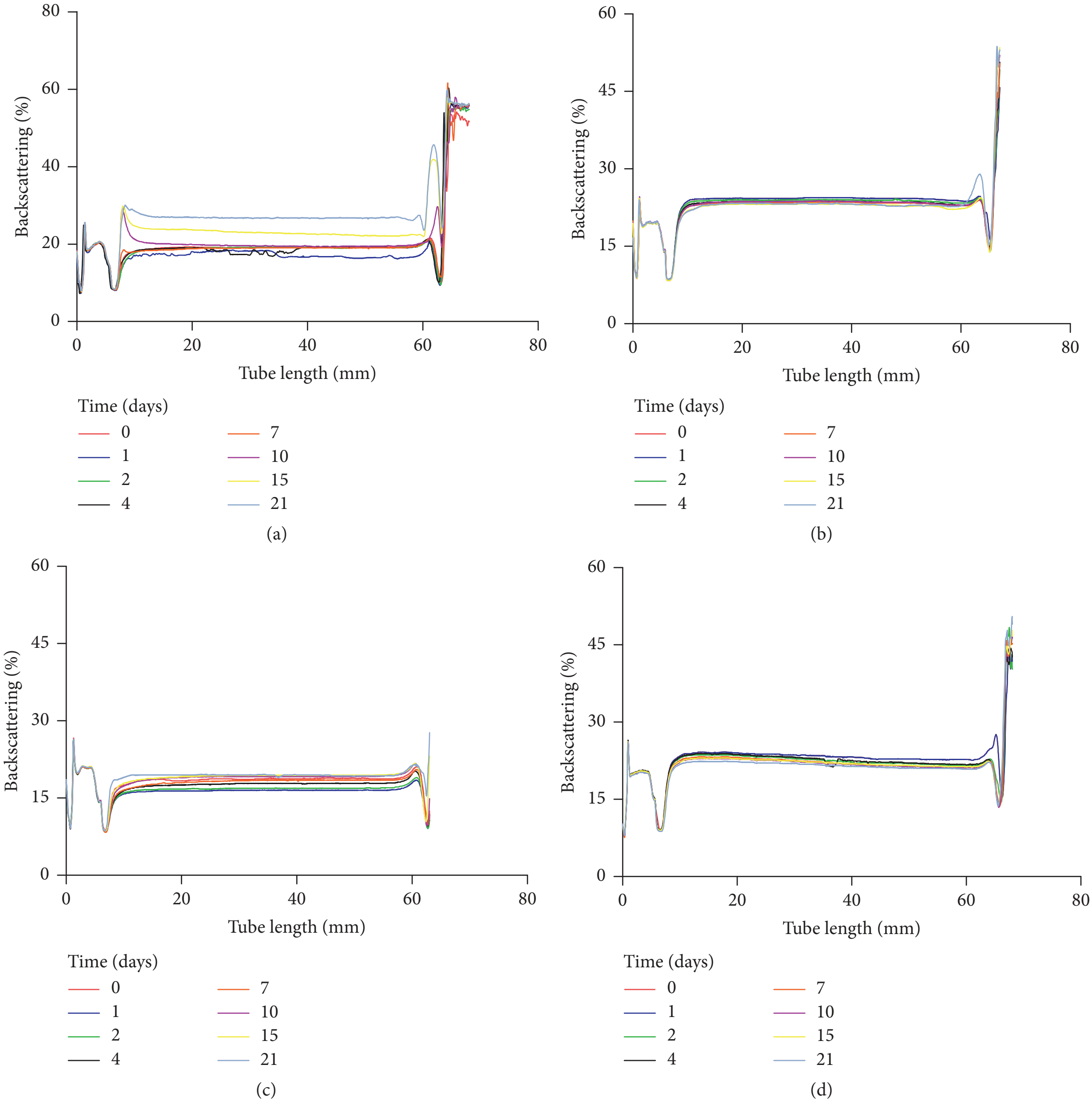
thinning flow behavior, having observed clear differences among them. Nanoemulsions with the highest oil and CMC concentration presented a higher degree of shear thinning behavior due principally to disruption of the spatial organization of oil droplets and CMC chains, being aligned in the direction of the shearing forces [21, 37]. This behavior is in agreement with other studies on emulsions with CMC and other polymers [13, 21, 38]. Nanoemulsions without CMC added showed a Newtonian flow behavior with flow behavior index values (n) around 1 (Table 3).

It is well known in the literature that CMC dispersions show a shear thinning behavior and its rheological properties have been adequately described by Ostwald-de Waele model [21, 39–41]. In this study, the flow curves of all CMC-based nanoemulsions were also successfully fitted to Ostwald-de Waele model with R^2 among 96.7–99.9%. ANOVA results of flow parameters showed a significant effect ($p < 0.05$) of factors studied (CMC and oil concentration) and the binary interaction between them on consistency index (K) and flow behavior index (n) values. As observed in Table 3, no significant differences were found between control nanoemulsions with different oil concentration, but when CMC was added, consistency and pseudoplasticity of samples increased. In general, K values increased significantly as increasing CMC and oil concentration, probably caused by the increase of resistance to flow due to polymer-solvent interaction. An increase of the effective volume fraction of the dispersed phase in these nanoemulsions, by an increase of oil concentration, results in a narrower distance between particles, which leads to packing of the oil droplets, and these interparticle interactions are stronger [13, 21]. Besides, the addition of CMC increased the viscosity of continuous phase since extended CMC chains start to overlap and entangle, resulting in a transient network structure increasing the resistance to flow [21, 42]. Regarding flow behavior index, by increasing CMC and oil concentration n values decreased significantly from 0.65 to 0.49, giving rise in a pseudoplastic behavior (Table 3). Pseudoplasticity represents an irreversible structural breakdown that may occur as a result of the spatial redistribution of the particles under a shear field by alignment of nonspherical particles (CMC) with the flow field or deformation and disruption of flocs (oil droplets) [16].

3.3. Stability. According to physical parameters results, nanoemulsions with 0.75% CMC at the two oil concentrations should be more stable to gravitational separation, since the physical basis of gravitational separation gives by Stokes' Law that the rate of droplet creaming should decrease as droplet size decreases, the density difference between phases decreases, and the aqueous phase viscosity increases [16]. In this case, samples with the highest CMC concentration presented higher consistency index (K) values (Table 3) than control samples and nanoemulsions with 0.5% CMC, and they showed the highest stability at two storage temperatures studied, observing phenomena of destabilization from day 15.

Figure 3 shows backscattering profiles (%) along the tube length for nanoemulsions prepared with different CMC and olive oil concentration at 5°C. Diverse destabilization processes were observed depending on CMC-olive oil ratio used, being all samples stable until 15 storage days. The emulsions containing 0.5 : 5 (% w : w) CMC-olive oil showed a high backscattering variation, making the most unstable emulsion (Figure 3(a)). This can be attributed to the high polydispersity index level of these samples (Table 2), which detected a small population of large particle that may cause instability by creaming during long-term storage [26]. Also, the instability phenomenon was attributed to physical mechanisms of destabilization as coalescence or flocculation, since according to the theory of multiple light scattering a variation of percentage of backscattering at tube meddle is associated with an increase in droplet size [43].

For the samples prepared with 0.5 : 15 (% w : w) CMC-olive oil, a decrease of the backscattering at the bottom of the tube was observed (Figures 3(a) and 3(b)), but one peak was observed in the top of the tube at the last time (21 days), which indicates gravitational phase separation of the emulsion by



(a)
(b)
(c)
(d)

Figure 3: Backscattering profiles as a function of the tube length after 21 days in quiescent conditions for nanoemulsions prepared at 5°C with (a) CMC-olive oil (0.5 : 5%), (b) CMC-olive oil (0.5 : 15%), (c) CMC-olive oil (0.75 : 5%), and (d) CMC-olive oil (0.75 : 15%).

creaming. Nevertheless, these results are consistent with the creaming index that showed low values. The observation may be explained by the consistence induced by the high level of olive oil in the emulsion formulation (consistency index values, Table 3).

The samples prepared with high CMC concentration showed the same behavior in function of olive oil in comparison with 0.5% CMC, but in general the highest stability values were obtained using a concentration of 0.75% CMC (Figures 3(c) and 3(d)). This result may demonstrate that, at high CMC concentration, the creaming stability was improved, which could be by an increase in the apparent viscosity due to the hydrocolloid contribution of the continuous aqueous phase. Previous researchers [44] have also reported that the addition of CMC to whey protein-based emulsion improved the physical stability. Other results indicated that the replacement of 0.3% (w/w) xanthan gum with 0.5% (w/w) CMC showed a significant increase in storage stability as compared to the control sample [17]. Also, as the CMC concentration increases, the droplets become smaller and the polydispersity was narrower, so that more stable emulsions can be expected [29].

The dependence of emulsion creaming index on the CMC-olive oil concentration in discontinuous phase at the different time and temperature is presented in Table 4. Practically all prepared emulsions storage at 5°C was more stable

Table 4: Creaming index of O/W nanoemulsions stabilized by CMC and with different olive oil concentration during 21 days of storage at 5°C and 20°C.

Storage time (day)	CMC : olive oil concentration (% w/w)				
	Control*	0.5 : 5	0.5 : 15	0.75 : 5	0.75 : 15
			Creaming index at 5°C		
0	1.65 ± 0.44^{aA}	3.39 ± 0.07^{bA}	0.72 ± 0.02^{aA}	1.87 ± 0.2^{aA}	1.13 ± 0.11^{aA}
15	3.54 ± 0.72^{aB}	5.68 ± 0.98^{aB}	5.82 ± 0.49^{aB}	5.63 ± 0.37^{aB}	5.15 ± 0.74^{aB}
21	4.17 ± 0.11^{cB}	6.60 ± 0.65^{bB}	20.73 ± 1.07^{aC}	5.68 ± 0.31^{bB}	6.48 ± 0.49^{bB}
			Creaming index at 20°C		
0	0.34 ± 0.02^{aA}	5.15 ± 0.61^{cA}	5.57 ± 0.67^{cA}	5.07 ± 0.86^{cA}	2.15 ± 0.69^{bA}
15	3.05 ± 0.18^{aB}	11.25 ± 0.85^{bB}	27.65 ± 0.73^{dB}	10.63 ± 0.85^{bB}	21.63 ± 0.63^{cB}
21	3.38 ± 0.08^{aB}	12.05 ± 0.45^{bB}	31.69 ± 0.74^{dC}	12.35 ± 0.65^{bC}	22.34 ± 1.07^{cB}

$^{a-c}$Significant ($p < 0.05$) differences among samples with different CMC-olive oil concentration from the same incubation time.

$^{A-C}$Significant ($p < 0.05$) differences among the same sample but with different incubation time.

*Control: without CMC added.

Table 5: Two-way ANOVA of lightness (L^*), red-green component (a^*), and yellow-blue component (b^*) for O/W nanoemulsions based on olive oil with different CMC concentrations. F and p values.

Parameters	Main effects				Interactions	
	A: oil concentration		B: CMC concentration		$A \times B$	
	F	p	F	p	F	p
L^*	5.13	0.04	80.37	<0.01	0.29	0.75
a^*	46.50	<0.01	3.44	0.38	0.25	0.78
b^*	119.42	<0.01	5.00	0.02	0.22	0.80

in the fresh form (storage time = 0) in comparison with 20°C. The difference among different O/W nanoemulsions became more obvious after 21 days of storage. Therefore, changes in the storage temperature affected the stability of emulsion because it can increase the droplet-droplet collision frequency, which can promote aggregation under conditions, where there is not a strong repulsion between the droplets [45]. Besides, an increase in the temperature can reduce the apparent viscosity promoting an increase in the creaming phenomenon [16].

All nanoemulsions stored at 5°C showed lower creaming index values at day 15, which is associated with very stable emulsions, because no serum layer separation in the tube was observed; therefore the creaming layer resulted slight. Conversely, the nanoemulsion containing 0.5 : 15 (% w : w) CMC-olive oil had the lowest stability among all these samples (Table 4). On the other hand, the storage of nanoemulsion at 20°C showed significant differences among CMC-olive oil samples ($p < 0.05$), where the highest creaming index value was obtained with 0.5 : 15 (% w : w) CMC-olive oil. When serum layer appears with time (i.e., the creaming index increased), it indicates a decrease in sedimentation stability or a clarification at the bottom of tube [29]. This indicated that using low olive oil concentration and high CMC concentration led to improvement of the physical stability to produce nanoemulsion. These samples showed the highest resistance against flocculation, coalescence, and phase separation in comparison with other samples, which can be due to the increase in the apparent viscosity when 0.75% CMC was added. This result is in agreement with a previous work [46], where it was observed that CMC addition reduced creaming index when 30% oil was added.

3.4. Color. Two-way ANOVA showed that only main factors had a significant effect ($p < 0.05$) on color parameters of different nanoemulsions (Table 5). L^* (lightness) and b^* (yellow-blue coordinate) were affected significantly ($p < 0.05$) by oil and CMC concentrations, while a^* values varied significantly ($p < 0.05$) with oil concentration. The addition of CMC into nanoemulsions decreased L^* values, but an increase of CMC concentration did not show effect on sample lightness (Table 6). This effect could be attributed to the structure of the CMC shape into nanoemulsions. The CMC could modify the spatial distribution of oil droplets, forming droplet aggregates which affect the scattering efficiency, since a decrease of the efficiency of light scattering decreases lightness of the emulsions [47]. The effect of hydrocolloids on color parameters is not clear in the literature, although hydrocolloids have been observed to modify instrumental color in some products. Chung et al. [48] showed in mixed colloidal dispersions based on emulsions that the lightness increased with increasing fat and surfactant content, while starch content had a little effect. In another study, Chung et al. [49] on mixed systems of locust bean gum (LBG) and emulsion obtained that the lightness decreased with increasing LBG concentration. Arancibia et al. [22] studied the effect of thickener type on color of semisolid desserts with different fat content and obtained that by increasing

TABLE 6: Mean values and significant differences of lightness (L^*), red-green component (a^*), and yellow-blue component (b^*) for O/W nanoemulsions based on olive oil with different CMC concentrations.

Oil concentration (% w/w)	CMC concentration (% w/w)	L^*	a^*	b^*
5	0	88.04 ± 0.26^{a}	-8.93 ± 1.27^{ab}	-33.40 ± 1.96^{b}
	0.5	80.90 ± 1.02^{b}	-7.42 ± 0.60^{a}	-29.99 ± 1.21^{b}
	0.75	81.13 ± 1.16^{b}	-7.57 ± 2.55^{a}	-29.92 ± 4.46^{b}
15	0	86.48 ± 1.02^{a}	-12.39 ± 3.02^{bc}	-21.80 ± 2.95^{a}
	0.5	80.26 ± 2.13^{b}	-13.42 ± 0.59^{c}	-19.20 ± 0.91^{a}
	0.75	79.95 ± 1.00^{b}	-13.50 ± 1.55^{c}	-17.39 ± 2.47^{a}

$^{a-c}$Means within a column with common superscripts did not differ significantly ($p > 0.05$).

CMC levels in skimmed-milk samples L^* increased; however, when starch levels increased in this type of samples, L^* values decreased.

Red-green (a^*) and yellow-blue (b^*) components varied with oil concentration, and the negative magnitude of these parameter values increased significantly as increasing oil concentration, at two CMC concentrations studied (Table 6). This effect can be due to the presence of pigments in the olive oil, as chlorophylls and carotenoids [50]. The concentration of chlorophylls and carotenoids in olive oil varies between 4.9–24.4 and 4.5–13.29 mg/kg respectively, depending on olive variety [51]. Chlorophyll pigments account for the greenness of this type of oils, while carotenoids account for their yellowness. In this case, the nanoemulsions with the highest oil concentration exhibited a more-intense green color than samples with 5% oil, while a decrease of yellowness with an increase of oil concentration was observed, which indicated that presence of carotenoids in olive oil did not affect the color of nanoemulsions. The increase of blueness can be due to the presence of chlorophyll "a" and chlorophyllide "a" that give a color bluish-green to olive oil [50].

To evaluate the effect of storage on color of nanoemulsions with CMC, the color differences between day 0 and different storage times (7, 14, and 21 days) were calculated as ΔE_{2000} color variation (Figure 4). At day 7, it was observed that samples with 0.75% CMC showed a higher color variation with values higher than 2, which indicated that color differences between these samples were noticeable to the human eye [32]. At day 14, the difference of color varied slightly in all nanoemulsions, although this increment was more evident in samples with the lowest oil concentration. At final storage time, variation of color depended principally on the emulsion stability since all samples showed creaming and phase separation affecting CIEDE_{2000} values. In general, almost all samples showed color differences noticeable to the human eye (values higher than 1.5).

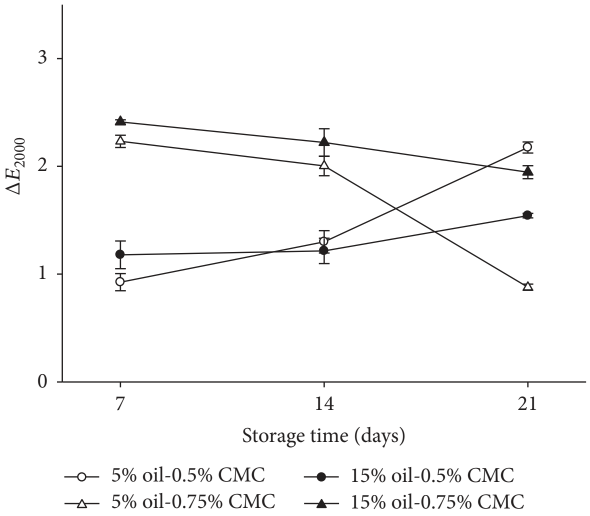


FIGURE 4: Difference of color (ΔE_{2000}) with respect to initial time (day 0) of storage at 5°C of different O/W nanoemulsions (5% oil: empty symbols, 15% oil: filled symbols, 0.5% CMC: circle, and 0.75% CMC: triangle).

4. Conclusions

The addition of CMC into nanoemulsions based on olive oil affected slightly the particle size of nanoemulsions, since it varied between 107 and 121 nm. All physical properties studied of nanoemulsions were depending on both oil and CMC concentration. Oil concentration affected principally color and flow properties of samples. However, the consistency obtained by the addition of CMC increased the stability of emulsions. Finally, the results showed that CMC could be applied as thickener into nanoemulsions based on olive oil, increasing their physical stability, but modifying their physical properties.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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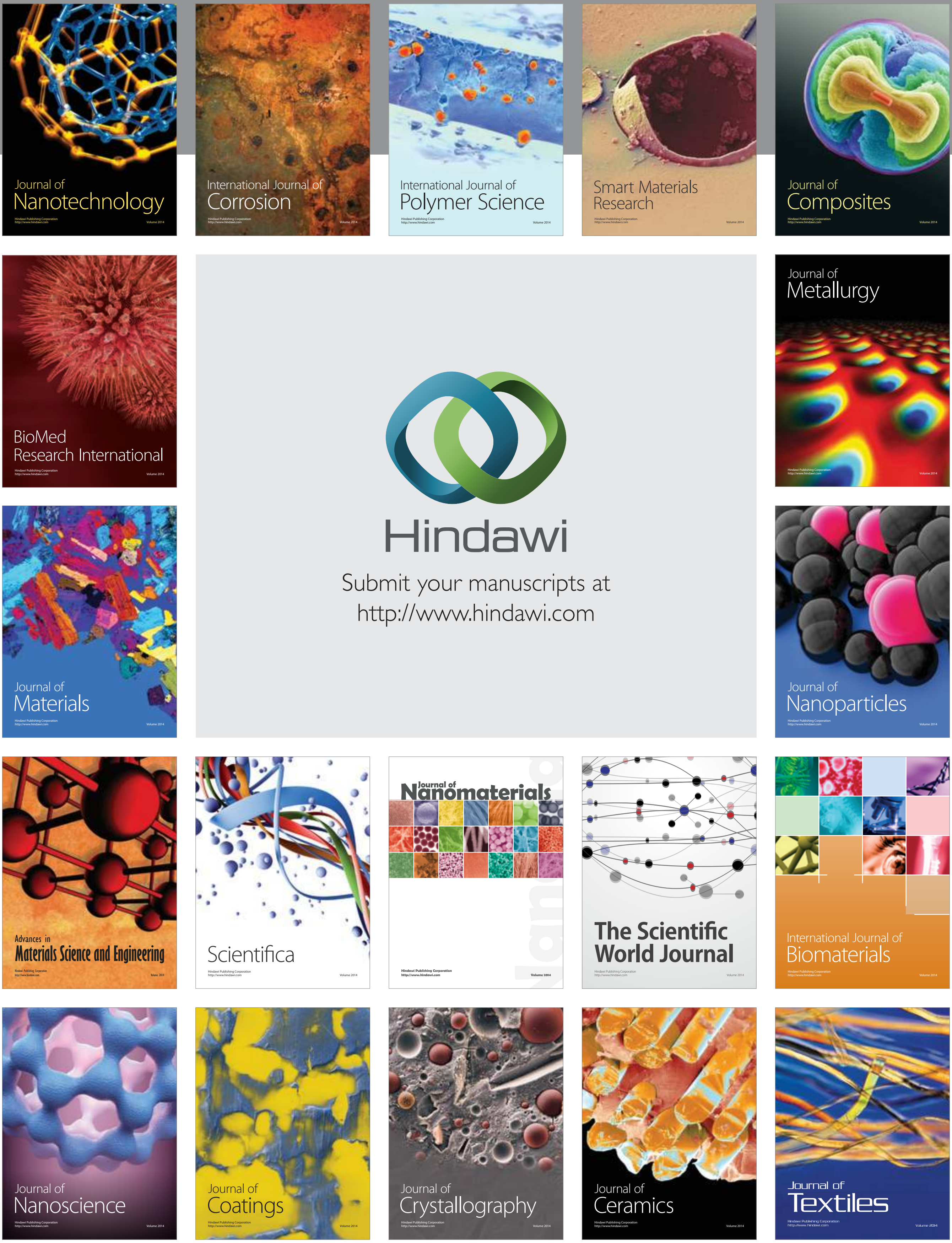
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