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Effect of process parameters on emulsion stability and droplet size of pomegranate oil-in-water

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SUMMARY: The development of efficient emulsion is essential and requires a good understanding of the parameters that govern the formation and stability of the emulsion. The droplet size significantly affects the stability of the emulsion. In this study, the stability of pomegranate oil-in-water emulsions (0.5 to 7.0% v/v) was investigated using various emulsifiers in terms of droplet size and instability index during 16 days of storage. The Mastersizer and Lumisizer were used to measure the droplet size and instability index. It was observed that the minimum droplet size was achieved by using 0.3% carboxy methyl cellulose (5.37 µm) and maximum with 1.0/2.5% whey protein/maltodextrin (24.26 µm). The Lumisizer results during storage revealed the higher emulsion stability of carboxy methyl cellulose due to smaller droplet size and high thickness as compared to other emulsions studied. The findings of the present study would

be useful for food applications to obtain fine and stable microcapsules.

KEYWORDS: *Emulsi iers; Emulsion; Optimization; Pomegranate oil; Stability*

RESUMEN: ***Efecto de los parámetros del proceso sobre la estabilidad de la emulsión y el tamaño de la gota de aceite de granada en agua.*** El desarrollo de una emulsión eficiente es esencial y requiere una buena comprensión de los parámetros que rigen la formación y la estabilidad de la emulsión. El tamaño de la gota afecta significativamente a la estabilidad de la emulsión. En este estudio, se investigó la estabilidad de las emulsiones de aceite de granada en agua (0,5 a 7,0% v/v) usando varios emulsionantes, en términos de tamaño de gota e índice de inestabilidad, durante 16 días de almacenamiento. El Mastersizer y el Lumisizer se usaron para medir el tamaño de gota y el índice de inestabilidad. Se observó que, el tamaño mínimo de gota se logró utilizando 0,3% de carboximetilcelulosa (5,37 µm) y el máximo (24,26 µm), con 1,0/2,5% de proteína de suero/maltodextrina Los resultados del Lumisizer, durante el almacenamiento, revelaron una mayor estabilidad de la emulsión de carboximetilcelulosa debido al tamaño de gota más pequeño y al alto espesor en comparación con otras emulsiones estudiadas. Los resultados del presente estudio se utilizarían en aplicaciones alimentarias para obtener microcápsulas finas y estables.

PALABRAS CLAVE: *Aceite de granada; Emulsión; Emulsionantes; Estabilidad; Optimización*

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1. INTRODUCTION

Emulsions are extensively utilized in various industrial applications but mainly used in the food industries. In the formulation of some food products such as yogurts, cream liqueurs, mayonnaise, ice cream, and salad dressings, emulsions play a very important role (Charcosset, 2009).

In general, emulsion is considered a heterogeneous composition, in which one immiscible liquid is dispersed into another liquid as droplets with diameters which surpass 0.1 mm (Bai *et al.,*2016). There are two predominant types of emulsions, one is oil-in-water (O/W) and the other is water-in-oil (W/O), while double or multiple emulsions types are O/W/O and W/O/W.

Emulsions are less thermodynamically stable; emulsions break up into two separate phases over time either quickly or slowly. The common mechanisms of the instability of emulsions are creaming, coalescence, Ostwald ripening, and flocculation (Tcholakova *et al.,* 2006). The shelf-life and quality of emulsions are highly affected by the aggregation of droplets. McClements, (2015) described that the properties of stable emulsions slowly change with time or show resistance to change. The increase in the droplet size of emulsions is one of the main reasons for the loss in stability (Silva *et al*., 2010). So, the instability of emulsions can be overcome by reducing the droplet size (McClements, 2015).

Krstonosic *et al.,* (2009) reported that the stability of the emulsion can also be increased with the addition of emulsifiers to decrease surface tension and avoid droplet flocculation by absorption on the surface of droplets. The main purpose of an emulsifier is to prevent the aggregation of newly formed droplets by forming a protective layer as well as decreasing interfacial tension. This results in stabilizing against coalescence (McClement, 2015).

Although the tools for characterizing emulsions are now well developed and the mechanisms of emulsification are reasonably understood, it is still difficult to predict the exact result of an emulsification process, since this is a combination of numerous parameters, including formulation and process variables.

Many micro molecule emulsifiers including Span, sodium dodecyl sulfate, Tweens, etc. and macromolecules of proteins and carbohydrates are added to foods and drugs to form stable emulsions (Hashtjin and Abbasi, 2015, Galooyak and Dabir, 2015). Forming a kinetically stable emulsion for a specific period to increase shelf-life is one of the main challenges of food product formulations.

Tween 20 is ester sorbitol and is widely used as an emulsifier in an oil-water emulsion with a hydrophile-lipophile balance (value 16) for food products. Usually, a low concentration of Tween 20 is considered safe while it is toxic at high concentrations. The carboxyl methyl cellulose (CMC) is taken as a thickener and emulsifier, as it imparts longer time emulsion stability and inhibits creaming by increasing the viscosity of the aqueous continuous phase (Dickinson, 2003). Whey protein (WP) is a dairy by-product and one of the most commonly studied emulsifiers used in polyunsaturated fatty acids (oils). Food proteins have excellent properties of foaming, gelling, and emulsifying as well as confering high nutrition to food products (Chen *et al*., 2006, Matalanis *et al*., 2011). Maltodextrin (MD) is also utilized with WP in combination as it is inexpensive, bland in flavor, and has low viscosity at high solids and prevents the emulsion from creaming or coalescence.

Many researchers have reported that a stable emulsion is a prerequisite for higher encapsulation efficiency, higher stability, smaller particles size, low surface oil and better retention of volatile components; while the opposite is true for the unstable emulsions (Minemoto, Hakamata, Adachi and Matsuno, 2002).

Over hundreds of years, the pomegranate has accompanied mankind as a symbol of life, longevity, health, knowledge, morality, and spirituality (Mackler *et al*., 2013). Pomegranate seeds contain around 3% of the total fruit weight and contain oil in the range of 12–20%. Pomegranate seed oil mainly consists of > 90% polyunsaturated fatty acids such as punicic acid (conjugated fatty acid), linolenic acid, and linoleic acid (Özgül-Yücel, 2005, Fadavi *et al*., 2006). As this oil is highly unsaturated, it easily oxidizes with heat, light, and air, which renders it unfit for edible consumption. It is feasible to add pomegranate oil into water emulsions for encapsulation to improve shelf-life. To the best of our knowledge no report has been published on the pomegranate oil-in-water emulsion.

Therefore, the present study aims at a better understanding of the effect of Tween 20, WP, and

MD as emulsifiers and stabilizers and CMC as a thickener, along with various parameters such as agitation speed, time, and concentration of pomegranate oil-in-water to prepare the most stable emulsion.

2. MATERIALS AND METHODS

2.1. Materials

Virgin pomegranate seed oil was purchased from Natural Source, USA. Various materials such as Tween-20, whey protein, maltodextrin DE=14, and carboxyl methylcellulose were purchased from Sigma Aldrich (Germany). Throughout the experimentations, deionized water from a Millipore (Leeds, MA) system was used.

2.2. Emulsion preparation

Four types of materials i.e. Tween 20, carboxyl methyl cellulose (CMC), whey protein (WP), and a combination of whey protein with maltodextrin (WP/MD) were selected as emulsifiers. To evaluate the effect of different parameters such as revolutions per minute (rpm) 5000 to 25000, time (1 to 5 min), Tween 20 concentration (0.5% to 7% w/v of 5 mL water) and amount of oil (0.5 to 7 g w/v), the emulsion stability and droplet size of pomegranate oil-in-water were optimized as shown in the flow chart in Figure 1. Optimized parameters were also further evaluated for the different concentrations of other emulsifiers on the basis of droplet size and instability index during 16 days of storage. First, the surfactant was dispersed into distilled water then the oil was mixed with magnetic agitation for 1 min. The mixture was then homogenized using a rotor-stator homogenizer.

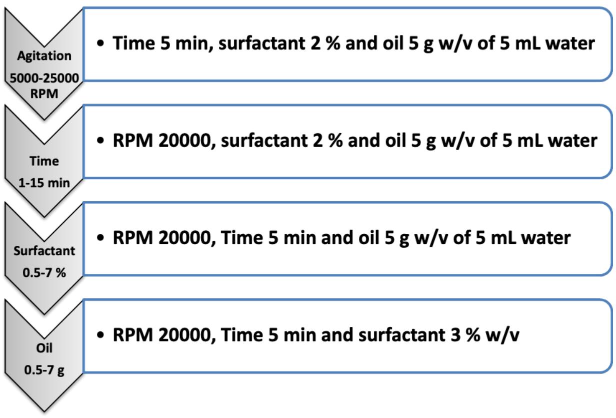

Figure 1. Flow chart of experimental design

2.3. Methods and instruments

A rotor-stator homogenizer, Ultra-Turrax type, IKA T 25 digital (Janke and Kunkel, Germany), with the following specification, was used for homogenization (25000 rpm, 800 W, Stator/rotor diameter: 25 mm (outside) and 18 mm (inside), the gap between rotor and stator 0.5 mm and immersion depth 40 – 165 mm).

2.4. Droplet size distribution

The mean droplet size was measured with a Mastersizer 2000 (Malvern, Worcestershire, UK) and expressed as the Sauter (surface average) diameter $d_{3,2.}$ The homogenized samples were analyzed after 1 h prior to preparation following a relative refractive index (1.465) of the dispersed phase (water). After adding drops of the emulsion into dispersion liquid when the obscuration index reached 15%, then the surface mean diameter $d_{3,2}$ and droplet size distribution was measured.

2.5. Centrifugal separation analysis Lumisizer

The LUMiSizer LS650 (Photocentric) multiple samples (Malvern, Worcestershire, UK) was used to understand the storage stability of the pomegranate emulsion that acts on the accelerated centrifugal mechanism. The assessment of emulsion stability (kinetic) requires a longer time, therefore, accelerated tests are used to mitigate this issue. Many techniques have been reported such as ultrasound, centrifugation, and turbidimetry or light scattering, and ultrasonic (Curt, 1994, Horozov and Binks, 2004) but failed in sufficient specificity and reliability in order to clarify the cause of destabilization. The most reliable and effective technique is Lumisizer, which directly measures the stability of emulsion (transmitted light intensity) in neat form. This instrument uses the Space and Time-resolved Extinction Profiles (STEP) technology, which measures transmitted light intensity over the entire sample length in a cuvette during centrifugation as a function of time (Lerche, 2002).

The instability index describes the reason for transmission clarification based on the separation of layer and particle size by accelerated gravitational force at a given time. It is a dimensionless number between 0 and 1 under the centrifugal field, where 0 (highest stability) represents no change in emulsion transmission or no separation, and 1 (lowest stability) indicates complete segregation of phases. Comparing instability indices of emulsion under accelerated gravitational field ultimately aids in a quick comparison of their shelf-life instead of waiting a long time at the earth's gravitation. A rectangular cuvette of polycarbonate of 298 mm was used to place 350 µL of sample and run on a centrifuge at 3000 rpm at 25 °C by applied laser wavelength (865 nm). The instability or separation index was calculated by SEPView software v 4.1.

2.6. Optical microscopy

The size of emulsions was observed with an optical microscope (BX41-Olympus, UK) connected to a digital camera (Cannon), Japan. One drop of the emulsion was kept on a glass slide and covered with a coverslip to avoid the mobilization of droplets. The droplet size was measured at room temperature with 20 x 100 magnification.

2.7. Statistical analysis

Statistical analysis of the data was carried out using Minitab16 USA software. Data were analyzed by analysis of variance (ANOVA) followed by the Tukey test ($p \leq 0.05$). Results are reported as mean ± (SD) of three replicates (each replicate corresponds to a different batch of emulsion).

3. RESULTS AND DISCUSSION

3.1. Optimization of agitation, time and amount of oil on emulsion droplet size

Initially, the effect of agitation (speed) on emulsion droplet size was carried out to get the smallest droplet size of emulsion on Mastersizer as shown in Figure 2a. According to our results, it was observed that at 20000 rpm, the smallest droplet size of emulsion was recorded at 20.61 µm. At higher agitation speed the size of emulsion droplet decreased with increasing agitation to break up droplets of oil from the large layer into smaller droplets that were coated with emulsifier or surfactant and formed stable or finer emulsion as reported by (McClements, 2015). It has been reported that high agitation speed promotes smaller oil droplet size with stable emulsion. Our results are also comparable to those of Li and Xiang (2019) for coconut oil, where a smaller droplet size of emulsion was found at 15000 rpm. Similarly, in another study, Yerramilli and Ghosh, (2017) observed similar results of the emulsion prepared with canola oil using 20000 rpm. Therefore, 20000 rpm was selected for further parameters.

After fixing or getting the smallest result for the droplet size with agitation at 20000, another variable factor effect of time on emulsion droplet size was examined. The effect of time was selected from 1 to 15 min from a total of five experiments (rpm 2000, Tween 20 2% w/v, oil 5g w/v of 5 mL water were constant) as shown in Figure 2b. With the smaller time period, larger emulsion droplets were produced from 1 to 2 min because of less time to break up oil layers into smaller droplets. With the greater time period (5 min) smaller droplets were obtained and beyond this, no significant effect on emulsion droplets size was observed as shown in Figure 2b. The same finding was reported by Bendjaballah *et al*., (2010) when mineral oil was homogenized at different periods to determine a smaller impact of emulsification time on finer droplet size.

According to McClement (2015), an emulsifier is a surface-active substance that is capable of adsorbing to an oil-water interface and protecting emulsion droplets from flocculation and/or coalescence. The effect of emulsifier on emulsion was checked by conducting 5 experiments using Tween 20 in the concentration range of 0.5% to 7% w/v in 5 mL water by keeping other parameters constant (oil 5 g, agitation 20000 rpm and time 5 min).

It was observed that the lower concentration of the surfactant could not form smaller droplets because it did not cover all oil droplets properly. Dickinson (2009) found that low concentrations of emulsifier (such as xanthan gum) had a destabilizing effect on emulsions due to a mechanism known as depletion flocculation. This mechanism is induced by the excess non-absorbing hydrocolloid and/or surfactant forming micelles as reported by Traynor *et al*., (2013). When surfactant concentration increased from 0.5% (29.38 µm) smaller droplets were obtained at 3% concentration and the smallest droplet size (17.25 µm) was found due to the structural forces inducing a repulsive energy barrier which enhances emulsion stability. At higher concentrations, 7% (18.36 µm) surfactant led to larger droplet size due to an increase

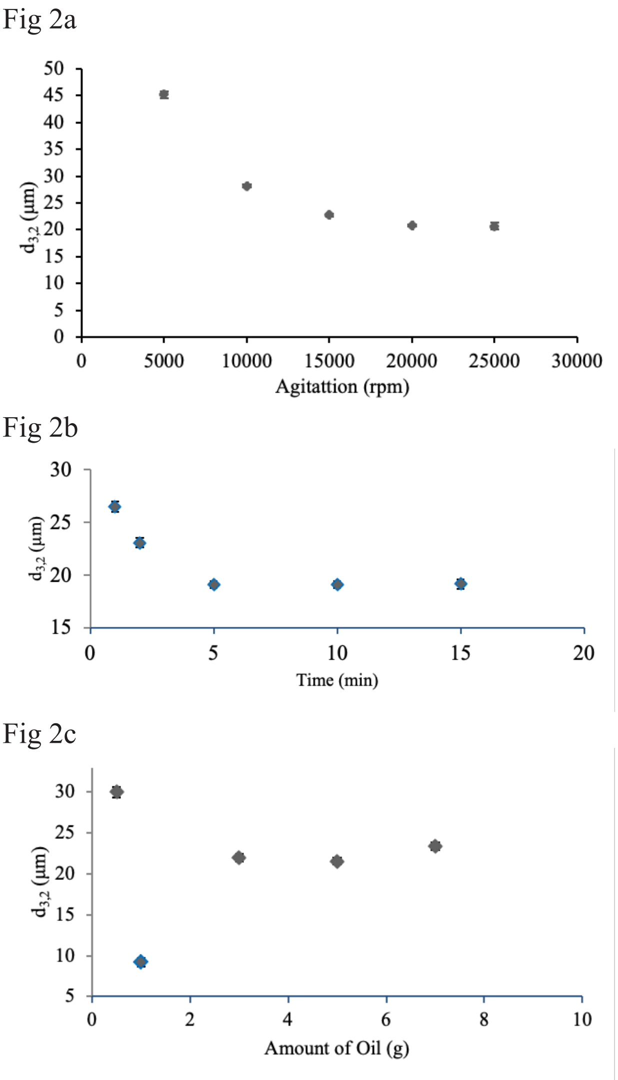

FIGURE 2. Optimization of process parameter on droplets size using Tween 20 as an Emusifier: (a) agitation, (b) time, (c) amount of oil. The values provided in the figures are the mean values of triplicate analyses with standard deviation.

in polydispersity in micellar size, which reduced emulsion stability and increased the size of droplets because of depletion flocculation, as reported earlier (Yerramilli and Ghosh, 2017). Our results also matched the findings of Jiao and Burgers (2003), who found that a higher concentration of Span 83 in mineral oil emulsion caused instability because of larger droplet size. Similarly, Bendjaballah *et al*., (2010) observed the same trend of droplet size on a concentration above 1%. In another study Traynor *et al*., (2013) reported that specific concentrations of xanthan gum with sunflower oil in emulsions had a stabilizing effect, but higher concentrations produced a destabilizing effect and instability due to an accelerated creaming process because of the promotion of droplet flocculation.

The oil volume ratio is very important for the size and stability of the droplets of emulsion. The high volume of oil ratio causes less oil to be entrapped by the emulsifier resulting in poor emulsion and larger droplets; while low oil concentrations were prone to destabilization by depletion flocculation due to the presence of non-adsorbing polysaccharides (Dickinson, 2003). As three variables were fixed, the most important factor, oil ratio with surfactant, was also analyzed. So, a different amount of oil from 0.5 g to 7 g was added to the water surfactant solution to check the droplet size. It was observed that at a lower level of oil such as 1 g smaller droplet size was produced, i.e. 16% of total 6 mL emulsion. On the other hand, higher oil concentrations led to higher mean diameters as shown in Figure 2c. The increase in oil concentration implied lower surfactant concentration since Tween 20 has emulsifying properties, therefore, a lower concentration of surfactant may have resulted in less efficient emulsification. Matalanis *et al*., (2011) found the finest droplet size at 20% of flaxseed oil emulsion, which is close to the present results. Similarly, the result of sunflower oil emulsion in xantham gum with a concentration of 19% oil showed finer droplet size as reported by Traynor *et al*., (2013). In contrast, Li and Xiang, 2019 observed smaller droplets at 5% coconut oil emulsion with ultrasound homogenization.

3.2. Use of different emulsifiers

After fixing all the parameters of pomegranate oil emulsion, different emulsifiers were used to obtain smaller droplet emulsion size, hence all the parameters were kept constant (such as rpm 2000, oil 1 g w/v of 5 mL distilled water, and time 5 min) except for the concentration of emulsifier, which varied, as shown in Table 1.

The pomegranate emulsion was also chosen as a stable emulsion at 3% of Tween 20, rpm 20000, oil 1 g w/v, time 5 min). The CMC was taken at very low concentrations from 0.1% to 0.5%. As 0.5% was too high a concentration and formed a viscous emulsion that could not be used for drying purposes and high droplet size was obtained. On the other hand, 0.3% concentration showed a smaller size of 5.3 µm of the droplet as shown in Table 1 with viscous emulsion. Arancibia *et al*., (2013) also reported the effect of CMC concentrations on the droplet size when 15% of the oil was emulsified with 0.3% CMC and obtained 9.21 µm. In another study, even smaller droplet size (2 µm) of olive oil with a 0.5% concentration of CMC was reported (Arancibia *et al*., 2016).

After optimization of the effect of CMC concentration on droplet size, WP was used as an emulsifier as well as a stabilizer to check the concentration effect (0.5 to 7.0%) on droplet size. It was observed that as the concentration of WP increased, smaller droplet size was obtained. When concentration further increased above 3%, the reverse trend in droplet size was observed with 10.60 µm and 12.26 µm, respectively, for 5 and 7% concentrations. It can be explained that the excess surface-active molecules that may accumulate on the droplet surface resulted in droplet destabilization and the larger droplet size. Furthermore, some structures can form micelles. At 3% emulsion concentration, the smallest droplet size was found to be 9.78 µm as shown in Table 1. The results obtained in this study also matched the work of Akhtar and Dickinson (2007), who reported 10 µm droplet size of WPI emulsion with triglyceride oil. A similar type of result (6.1 µm) was also found by Hebishy *et al*., (2017) when 4% WPI in 30% sunflower/olive oil was homogenized according to the conventional mill method.

A combination of emulsions was also used to measure emulsion droplet size in the continuation of a single emulsifier. The concentrations of WP and MD remained constant (3.5% of total emulsion) while varying the concentrations of both individually to check the effect on droplet size as shown in Table 1. It was observed that lower emulsion droplet size was obtained when the concentrations of WP and MD were kept at 2.5% and 1%, respectively; while other studied combinations produced higher emulsion droplet size (Table 1). In contrast to our results, Akhtar and Dickinson (2007) reported lower emulsion droplet size (1 µm) with a combination of WP/MD in a ratio of 1:2 than using alone WP.

The droplet size of various emulsions was also confirmed with the optical microscope, which supported our claim that smaller droplets were thicker in CMC images than WPC-MD, and larger droplets, but less thick in WP and Tween 20 as shown in Figure 3.

Table 1. Effect of concentration of emulsions on droplet size. The experiment was done in triplicate and the Tukey test was used for the comparison of mean values ($p<0.05$). Letters a–e indicates a significant difference among a different concentration of the same emulsifier.

Emulsions	Concentration (%)	$d_{3,2}$ (µm) Mean±SD
Tween 20	0.5	15.39±0.17[a]
	1.0	12.78±0.20[b]
	3.0	10.27±0.35[e]
	5.0	11.56±0.28[c]
	7.0	11.20±0.45[d]
CMC	0.1	13.03±0.18[a]
	0.2	9.72±0.65[c]
	0.3	5.37±0.81[e]
	0.4	10.63±0.42[b]
	0.5	7.45±0.33[d]
WP	0.5	14.33±0.11[a]
	1.0	10.72±0.50[d]
	3.0	9.54±0.74[e]
	5.0	11.60±0.68[c]
	7.0	12.26±0.85[b]
WP/MD	1.0/2.5	24.28±0.93[a]
	1.5/2.0	18.51±0.26[b]
	2.0/1.5	15.83±0.48[c]
	2.5/1.0	9.12±0.39[e]
	1.75/1.75	12.65±0.57[d]

CMC, WP, WP-MD, $d_{3,2}$, and SD stand for carboxy methyl cellulose, whey protein, whey protein/maltodextrin, sauter mean diameter, and standard deviation, respectively

3.3. Emulsion stability measurement during storage

The stable emulsion must prevent change in the size of droplets during storage with time and it is an immense challenge for the scientific community and industrial production. The results of our stability measurements on emulsions containing CMC, Tween 20, WP, WP-MD were checked on different days with the Lumisizer. As can be seen in Figure 4, the lower instability index value of CMC showed higher stability and less separation of emulsion than using WP-MD; while higher instability index values were observed for WP and Tween 20, respectively, which led to instability, creaming, and coalescence of emulsions.

Emulsion stability is generally linked to mean droplet diameter and variation in rheological properties. When $d_{3,2}$ is small, viscosity variation is slow and the emulsion is more stable, as described elsewhere (Tadros, 2004; Sánchez *et al*., 1998). Smaller droplets and narrow size distribution of CMC were observed (Figure 4) because of high molecular weight and pseudo plastic behavior compared to WP/MD, and WP; and Tween 20 can have low shear thinning behavior.

Furthermore, we also checked the storage stability of emulsion for 16 days and measured droplet size on the Mastersizer. As days increased from 1 to 16, the size of droplets increased, which led to instability of emulsion and formation of creaming and coalescence. The CMC emulsion has more thickness and lower droplet size on the first day at 5.37 µm. Hence, it showed more stability than other emulsions, but its droplet size increased gradually with the passing of days and finally reached at 52.36 µm on day 16. While other emulsions showed a comparatively higher increase in droplet size as days passed because of less thickness and higher droplet size on initial days. In high viscosity, the negative or positive charge on molecules causes them to repel each other and does not allow movement of droplets easily so fewer chances for coalescence and creaming exist (McClements, 2015). WP-MD emulsion showed the second best stability due to high thickness, lower droplet size, and a combination of two emulsifiers. The decreasing stability order of emulsions was observed as: CMC> WP/MD > WP > Tween 20, as shown in Table 2.

4. CONCLUSIONS

This is the very first time we have reported pomegranate oil-in-water emulsions stabilized by surfactants and emulsifiers to get finer droplets. The experimental conditions allowed us to form very stable emulsions with very small droplet size $d_{3,2}$ (5.37 µm) and narrow distribution with a single surfactant. The Lumisizer results during storage also revealed higher emulsion stability with CMC. The higher stability of emulsion (0.3% CMC) can be attributed to the competing role of negative repulsive versus attractive depletion forces such as the anionic polysaccharide structure of CMC. These stable emulsions would be utilized for related food applications.

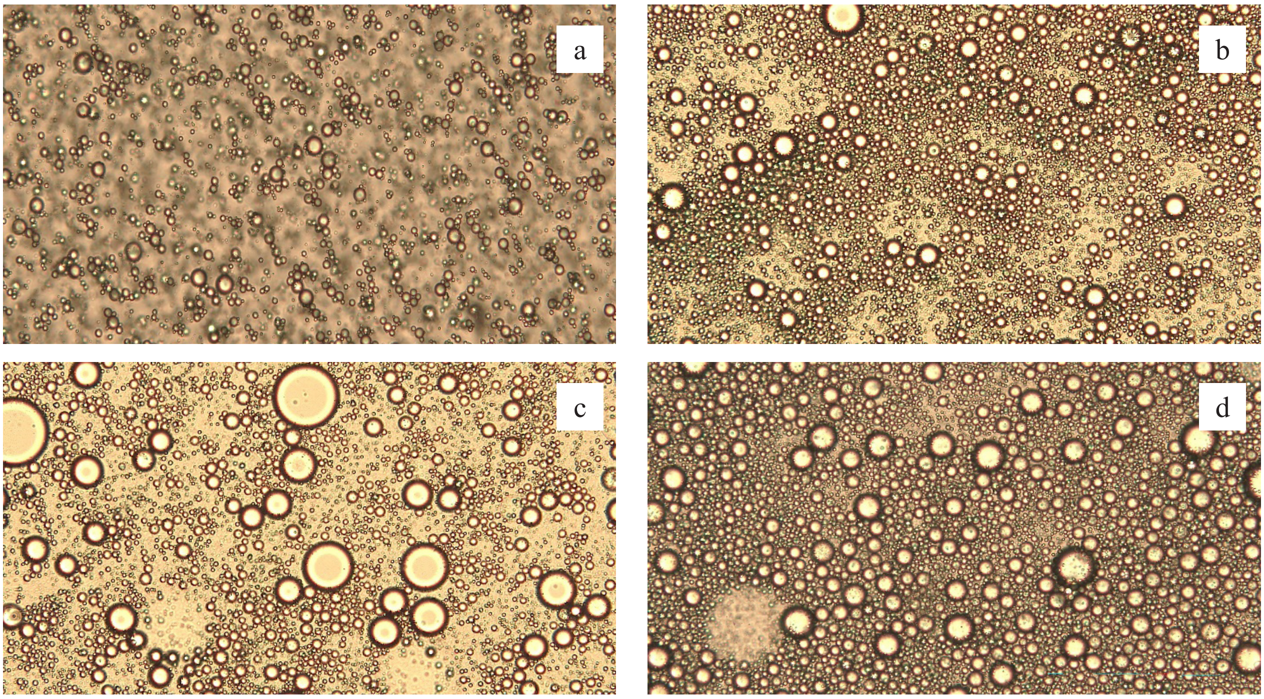

FIGURE 3. Optical microscope images of emulsion at 20×100 magnification, (a) Tween 20, (b) CMC, (c) WP, (d) WP/MD

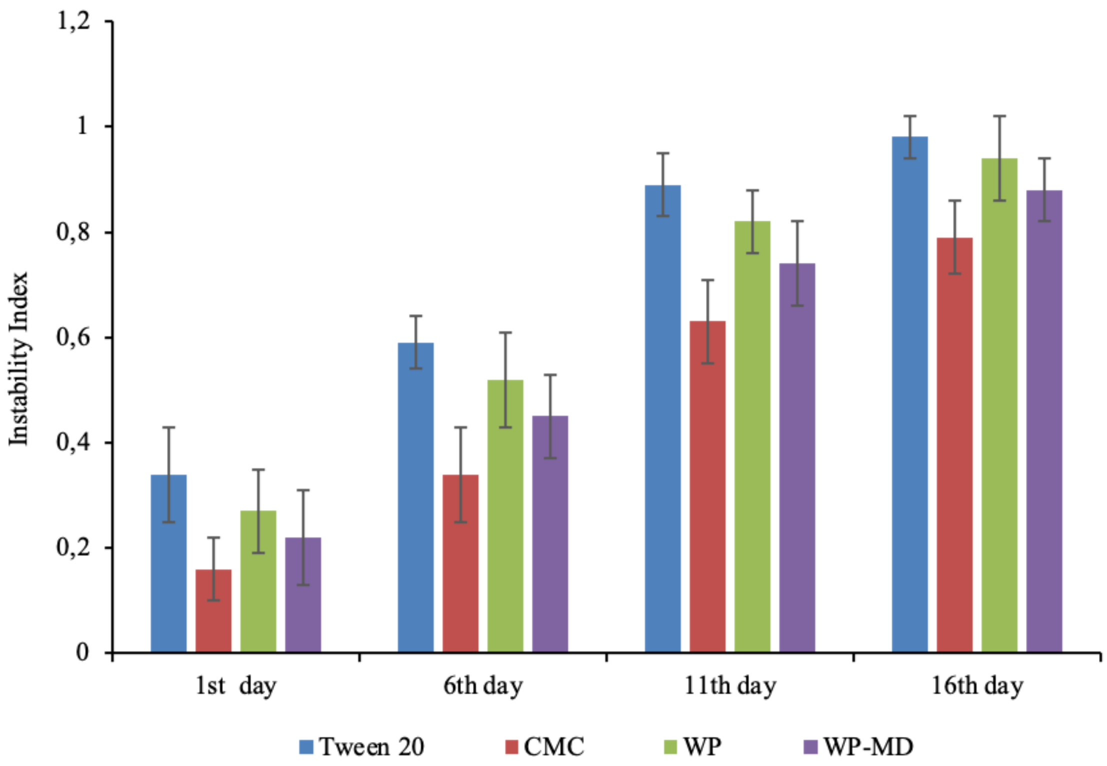

Figure 4. Instability index profile of emulsions during storage. The values provided in the figure are the mean values of triplicate analyses with standard deviation

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Conflict of Interest

The authors have declared no conflict of interest.

Table 2. Effect of storage on the stability of various emulsions on droplet size. The experiment was done in triplicate and the Tukey test was used for the comparison of mean values ($p < 0.05$).

Emulsion	$d_{3,2}$ (μm) ±SD Days			
	1	6	11	16
Tween 20	10.27±0.35^{d}	32.21±0.14^{c}	54.32±0.33^{b}	106.87±0.72^{a}
CMC	5.37±0.81^{d}	17.56±0.47^{c}	31.21±0.27^{b}	52.36±0.64^{a}
WP	9.54±0.74^{d}	21.43±0.69^{c}	38.65±0.46^{b}	78.29±0.85^{a}
WP-MD	9.12±0.39^{d}	19.21±0.82^{c}	36.32±0.28^{b}	57.87±0.94^{a}

CMC, WP, WP-MD, d32, and SD stand for carboxy methyl cellulose, whey protein, whey protein-maltodextrin, sauter mean diameter, and standard deviation, respectively.

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