



## Optimization of Oleofoam and Protein-Polysaccharide Ratios for Enhanced Physicochemical Characteristics of A/O/W Double Emulsion: Potential Applications in the Food Industry

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### Abstract

Preparing air-in-oil-in-water (A/O/W) double emulsion involves two key steps: oleofoam formation and dispersion of the oleofoam in an aqueous solution containing protein as an emulsifier and hydrocolloid as a thickening agent. This study aimed to investigate the effect of oleofoam level and varying concentrations of protein-polysaccharide ratios on the thermal stability, encapsulation yield and rheological properties of A/O/W double emulsion. An oleofoam was obtained using a lipophilic emulsifier (distilled monoglyceride MG) and sunflower oil at 5°C with maximum stability. Two levels of oleofoam (20% and 25 wt %) were added to an aqueous solution containing different concentrations of sodium caseinate (SC) (5, 8, and 10 wt %) and kappa carrageenan (KC) (0.4 and 0.8 wt %). Results indicate that oleofoam level did not significantly affect air encapsulation efficiency and particle size, while protein-polysaccharide ratios could significantly impact all properties of A/O/W double emulsion. Increasing the concentration of sodium caseinate and kappa carrageenan improved thermal stability and encapsulation yield while simultaneously reducing particle size. All A/O/W emulsions exhibited shear thinning behavior among the range of shear rates studied, indicating significant potential for food applications.

**Keywords:** A/O/W double emulsion, Oleofoam, Protein-polysaccharide ratios, Rheological properties



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## Introduction

Oleof foam is a type of foam that has attracted increasing attention from researchers and industry professionals in recent years. This unique foam is formed by the stabilization of air in the oil phase using oil-soluble surfactant molecules like fats (triglycerides) and fatty acids/alcohols/esters, which can form solid crystals with varying shapes and structures. These crystals can interact with the surrounding air medium to create a strong network of interlocking crystal particles, resulting in a foam-like structure (Binks & Vishal, 2021). Oleofoams have a wide range of properties that make them a promising candidate for various applications, from food products to cosmetics and health care; it is a cheap novel system with low-fat content and calories, pleasant texture, long-term stability and microbial spoilage resistance (Fameau & Binks, 2021; Murray, 2020). This structured oil helps to follow the growing trend of creating "clean label" products (Fameau & Binks, 2021) and to decrease the utilization of saturated or trans-fatty acids in food products (Qiu *et al.*, 2021). Recently, oleofoam has successfully been used as an alternative fat to produce low density sponge cakes (Wetlaufer & Floter, 2022) and aerated mayonnaise sauces (Saremnejad *et al.*, 2019).

Foamed emulsions (emuleofoam) come in different types, depending on the continuous phase surrounding the bubbles. These include aqueous foams with no oil, water-in-oil emulsions, bicontinuous oil and water phases, and oleo foams (Salonen, 2020). Recently a new type of air-in-oil-in-water (A/O/W) double emulsion has been investigated. A two-step preparation process is employed to produce A/O/W double emulsions; the initial step involves the aeration of the oil phase that contains endogenous crystals which come from the oleogelator. Ensuring an optimal solid fat content is essential for the stabilization of air bubbles while maintaining the desired fluidity of the oil phase in the following step. Subsequently, the oleofoam is progressively incorporated in an external aqueous phase containing an emulsion stabilizer and

thickening agent at such large concentrations (Goibier *et al.*, 2019). Food scientists will find these novel emulsions highly intriguing as they can decrease fat intake.

Previous studies have investigated the optimization of A/O/W emulsions by employing various emulsifiers and thickeners. Burn *et al.* (2015) successfully developed a stable A/O/W emulsion using oleofoam with rapeseed oil, mono- and diglycerides, and hydrophilic emulsifiers such as sodium caseinate or gum Arabic. Goibier *et al.* (2019) focused on enhancing A/O/W emulsions by utilizing an oleofoam composed of anhydrous milk fat and sodium caseinate as the sole emulsifier. Qiu *et al.* (2021) achieved the stabilization of oleofoam containing medium-long chain diacylglycerol and  $\beta$ -sitosterol through the incorporation of gum Arabic and xanthan gum. These studies collectively contributed to advancing the quality and stability of A/O/W emulsions for diverse applications through the strategic selection and combination of emulsifiers and thickeners, but they did not clarify the effect of each component (oleofoam, emulsifier and thickener) on the emulsion properties.

Emulsions' stability depends on synthetic surfactants or natural molecules (biopolymers) like proteins and polysaccharides (Seddari & Moulai-Mostefa, 2015). The intermolecular interaction between protein and polysaccharide plays a vital role in enhancing the stability of the A/O/W emulsion. The mechanism behind A/O/W double emulsions phenomenon stability involves biopolymer adsorption, usually protein, followed by adding polysaccharides. The polysaccharide does not interact with the adsorbed biopolymer, but it increases the viscosity of the continuous aqueous phase. In the process of emulsification, the protein tends to create small droplets. In contrast, the polysaccharide produces a more robust and compact network (Paraskevopoulou *et al.*, 2005), stabilizing the A/O/W emulsion. The main objective of this study was to explore the potential of A/O/W double emulsions as potential fat substitutes in food applications. To

achieve this, a series of double emulsions were prepared by systematically varying the concentrations of sodium caseinate (5, 8, and 10 wt. %) and kappa carrageenan (0.4 and 0.8 wt. %) within two different levels of oleofoam (20 and 25 wt. %). The study was focused on investigating the impact of these three factors on various properties of the double emulsions, including their microstructure, bubble size distributions, rheological behavior, and thermal characteristics. By analyzing these properties, we aimed to gain scientific insights into the suitability of these double emulsions as potential fat substitutes in food products.

## Materials and Methods

### Materials

Distilled monoglyceride (MG) was obtained from Palsgaard, with a melting point of 65 °C. The sunflower oil used in the study was purchased from the local market in Iran and has a relative density of 0.918 and a viscosity of 58 cP at 20°C. Dodecyl sulfate (SDS) (C<sub>12</sub>H<sub>25</sub>O<sub>4</sub>SNa), Tween 80, and sodium azide (NaN<sub>3</sub>) were procured from Sigma-Aldrich, France. Sodium caseinate (SC) from the Dutch DMW Company and kappa-carrageenan (KC) from Negin Khorak Pars in Iran were also obtained for the research.

### Oleofoam formation

Monoglycerides are amphiphilic molecules (they have both hydrophilic and hydrophobic regions). The glycerol backbone is hydrophilic, while the fatty acid chain is hydrophobic. This unique structure allows monoglycerides to interact with different components in food systems. They also can form a protective layer around air bubbles, preventing coalescence and improving the stability of foams and aerated products (Binks & Vishal, 2021). The oleofoams were prepared by dissolving 10 wt. % of monoglyceride (MG) in sunflower oil at 80°C for 5 minutes and then cooling it to room temperature. The resulting mixture was whipped at two different temperatures (25°C and 5°C) using a 5-speed mixer (Gosonic, model No. GHM-818, 250W, China) at the

highest speed for 30 minutes (Saremnejad *et al.*, 2019).

### Oleofoam characterization

The oleofoam was observed using an Olympus BX41 microscope (Olympus BX41, Japan) equipped with a digital camera (Canon EOS 1000D); then, about 500 bubbles from three pictures were used to measure the bubble size and distribution using image analysis software (ImageJ 1.50f for Windows). Foamability parameter was measured in the production time using Eq. (1) (Saremnejad *et al.*, 2019). The over-run of the oleofoam is calculated using Eq. (2) (Liu *et al.*, 2021).

Foamability = (The volume of foam / the initial volume of liquid) × 100 (1)

% Overrun =  $\frac{(V_{oil+air}) - (V_{oil})}{(V_{oil})} * 100$  (2)

Where (V<sub>oil</sub>+V<sub>air</sub>) equals the volume of oil plus entrapped air bubbles, (V<sub>oil</sub>) is the volume of the oil which was warmed to (80 ± 2°C) in order to fully release air bubbles.

### Oil drainage

Oil drainage from oleofoam samples was measured after storage at 25°C for 1 h, 24 h, 72 h, 7 days, and 28 days. Samples of approximately 10 mL were placed in a centrifugal tube. After each storage period, the amount of drained oil was measured in triplicate and calculated using Eq. 3 (Alhasan *et al.*, 2023).

Oil drainage (%) = V (drained oil) / V (oleofoam) × 100 (3)

### A/O/W double emulsion formation

To prepare sodium caseinate (SC) emulsifiers, controlled concentrations of emulsifiers 5%, 8%, and 10% were dissolved in water containing sodium azide via magnetic stirring at room temperature. Kappa-carrageenan (KC) with percentages of 0.4% and 0.8% were dissolved in distilled water at 8Δ°C using magnetic stirring for 30 minutes. The resulting solutions were stored for 12 hours at 5°C to complete hydration. The A/O/W double emulsion was prepared by combining oleofoam solutions with different concentrations of 10

%MG (20% and 25%) prepared at a controlled temperature of 5°C. The oleofoam solutions were mixed with the aqueous phase (SC-KC) in a 1:1 weight ratio, following the method described by (Brun *et al.*, 2015) and (Qiu *et al.*, 2021) with modifications. The mixed solutions were then sheared using a digital high-shear mixer dispersion; It must be noted that a cylindrical probe with a diameter of 59.7 mm (bob) inside another cylinder (cab) with a diameter of 65.7 mm was used to achieve proper emulsion formation and maintain the integrity of the multiple structures at a speed of 1500 rpm for 60 se. at 25°C to reduce the droplet size in the final multiple A/O/W emulsions. After shearing, the resulting emulsion was promptly refrigerated for further analysis.

#### A/O/W double emulsion characterization

##### Encapsulation yield of air bubbles

The total content of oil droplets containing air bubbles was determined after mixing using Eq. (4) (Goibier *et al.*, 2019).

$$EY = \frac{V1-V2}{V_{foam \times overrun}} \times 100 \quad (4)$$

Where V1 is the volume of the emulsions after centrifuging, V2 is the volume of the emulsion in the absence of air bubbles, and V foam is the foam volume initially introduced in the double emulsion.

##### Optical microscope observation

The globule size distribution of A/O/W emulsions was measured using a Laser Particle Sizer ANALYSETTE (Fritsch, Germany), and the optical microscope utilized in the study was an (Olympus BX41, Japan), which was equipped with a Canon EOS 1000D digital camera. Photomicrographs were captured to visualize the oil droplets containing air bubbles in the A/O/W emulsions. Certain emulsions had high viscosity, necessitating their preparation by previous research (Goibier *et al.*, 2019).

##### Thermal Stability

The thermal stability of the A/O/W emulsions was analyzed according to the method of Liu *et al.* (2021) with slight

modifications. Ten mL of each sample (F0) of The A/O/W emulsions were then transferred into tightly sealed tubes with plastic caps and maintained at 75 °C for 5 min. The oil that separated from the emulsion (F1) was subsequently measured. The stability of the emulsion was calculated using the following equation (5):

$$\text{Stability} = (F1 / F0) \times 100 \quad (5)$$

##### Rheological properties

A Bohlin rotational viscometer (Bohlin Model Visco 88, Bohlin instruments, UK) was employed to evaluate the rheological properties of the samples. The measurements were conducted at a constant shear rate of 50 (s<sup>-1</sup>) and over a range of 14-400 (s<sup>-1</sup>) at 25 °C.

##### Statistical analysis

Experiments were performed in triplicate. The collected data were analyzed by one-way analysis of variance (one-way ANOVA). Significant differences in means were compared using Duncan's test at a 5 % significance level using the SPSS 16.0 statistical software (SPSS Inc., Chicago, USA)

## Results and Discussion

### Oleofoam

#### Foamability

The foamability of oleogels, prepared with a 10% of MG at 25°C and 5°C, was evaluated in this study. Fig. 1, Demonstrates that the sample prepared at 5°C exhibited higher formability and overrun percentage compared to the sample prepared at 25°C throughout the preparation process. This indicates that the formability and overrun of the oleogels were significantly influenced by the temperature of the preparation. Lower temperature has been observed to have a stabilizing effect on air bubbles in oleofoams, as it leads to higher solid fat content. Mishra *et al.* (2020) reported that at lower temperatures, specifically 5°C, there was an increase in overrun. This increase in overrun can be attributed to the formation of  $\alpha$  crystals, which subsequently undergo a transformation into  $\beta$  crystals during the aeration process.

These findings highlight the role of temperature in influencing the crystal structure and stability of oleofoams. In this study, both samples demonstrated high overrun, with the sample prepared at 5°C exhibiting an overrun value of 140%, higher than the other sample which had an overrun value of 120%. This result indicates that lower temperatures contribute to an increased overrun, likely due to variations in crystal number and shape within the continuous oil phase, ultimately enhancing foaming ability (Himawan *et al.*, 2006). These findings align with the results of Liu & Binks (2021), who investigated the production of oleofoam using olive and peanut oils, and found that the lowest temperature yielded the highest foamability. Similarly, Lei *et al.* (2020) achieved an overrun of approximately 70% for a whipped 10 wt% DAG-based oleogel. They accomplished this by rapidly cooling the sample from  $80 \pm 2^\circ\text{C}$  to  $5 \pm 2^\circ\text{C}$ , followed by gradual heating to  $25^\circ\text{C}$ .

The whipping time also influences the foamability and overrun of the oleofoam. The

foam volume significantly increased in the first 5 minutes of the total aeration time (30 minutes) due to the high adsorption of MG crystals at the oil-air interface, allowing for the incorporation of more air. However, an excessively long whipping time for the sample prepared at 5°C resulted in decreased overrun. This occurrence can be attributed to the shearing forces encountered during the mixing process at high viscosity. These shearing forces have the potential to rupture the bubble film formed by the MG, leading to the collapse of the bubbles. The components of the MG film are crucial for providing structural stability and support the air bubbles within the matrix. Damage to these film components can ultimately result in the collapse of the foam. Similar results have been reported for the whipping of sunflower oleofoams prepared with different concentrations of mono and diglyceride (Saremnejad *et al.*, 2019) and high-oleic acid sunflower oleofoams prepared with myristic acid at different concentrations (Liu & Binks, 2021).

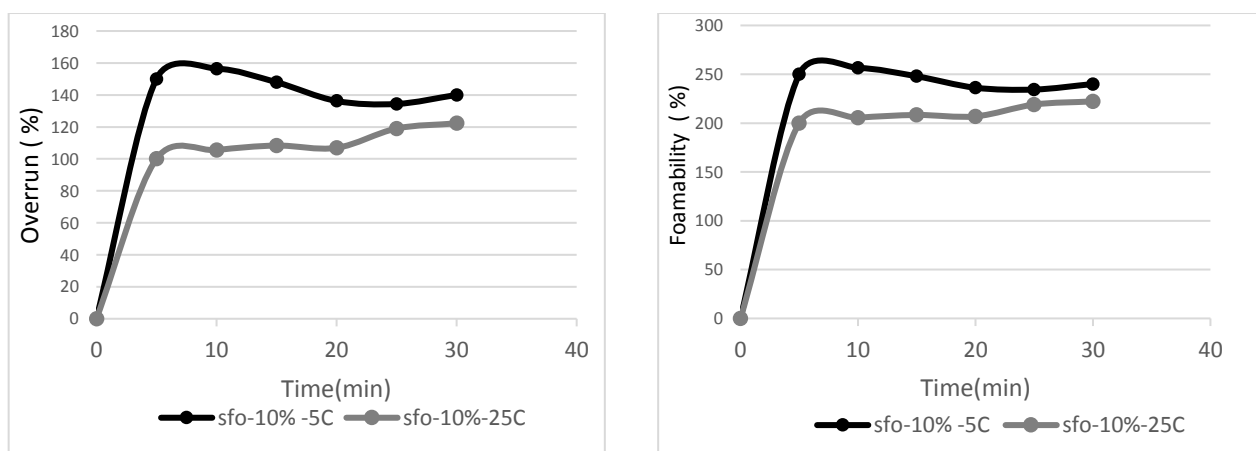


Fig. 1. Variation of the overrun and formability of the oleofoam based on monoglyceride (MG) measured each 5 min during the aeration

### Optical microscope oleofoam observation

In the oleofoam micrograph (Fig. 2a and b), most bubbles are spherical due to the adsorption of surfactant crystals that hindered shape relaxation, in line with the findings of (Saremnejad *et al.*, 2019; Liu & Binks, 2021). The two systems exhibited different size

distributions due to variations in their preparing temperatures. As the temperature decreased, a narrower distribution and smaller bubble sizes were observed. The average bubble size in the sample prepared at room temperature was 20  $\mu\text{m}$ , which decreased to 10  $\mu\text{m}$  in the samples prepared at low temperature, which might be



attributed to several factors. Firstly, at lower temperatures, the viscosity of the oleofoam increases (Wildmoser et al., 2004). This higher viscosity restricts the mobility of the air bubbles, preventing their coalescence and leading to a narrower size distribution. The increased viscosity also hinders the growth of bubbles, resulting in smaller bubble sizes. Secondly, the higher solid fat content at lower temperatures promotes the stabilization of air cells within the oleofoam matrix. The solid fat acts as a structuring agent and are thus surface-active (Binks & Marinopoulos, 2017) providing stability and support to the air bubbles. This structural stability helps to maintain the integrity of the bubbles, preventing them from merging or collapsing and contributing to the smaller bubble size and narrower distribution. Lastly, the slower diffusion of gas at lower temperatures may also contribute to the formation of smaller bubbles. The reduced diffusion rate limits the expansion of the bubbles, resulting in smaller sizes. Overall, the combination of higher viscosity, solid fat content, and slower gas diffusion at low temperatures contributes to the observed narrow distribution and smaller size of bubbles in the oleofoam, the effect of temperature

production on particle size or distributions has not been studied yet.

Fig. 3 Indicates microscopic images of oleofoam samples at different temperatures. It shows an apparent decrease in bubble size as the temperature decreases which caused more stable oleofoam due to the fact that at lower temperatures, the interfacial tension between the foam bubbles and the surrounding medium increases, making it more difficult for the droplets to coalesce and merge. As a result, the overall particle size of the emulsion tends to decrease.

### Oleofoam stability

For production of double emulsion, the oleofoam must exhibit high stability against oil loss. The foam stability as a function of production temperature at 25°C is shown in Fig. 4. Samples prepared at 25°C showed oil drainage within less than 24 hours, which continued for 28 days. The most stable foam was obtained from samples prepared at 5°C. This can be attributed to the size and homogeneity of the initial bubble size distribution, which play a crucial role in the stability of oleofoam.

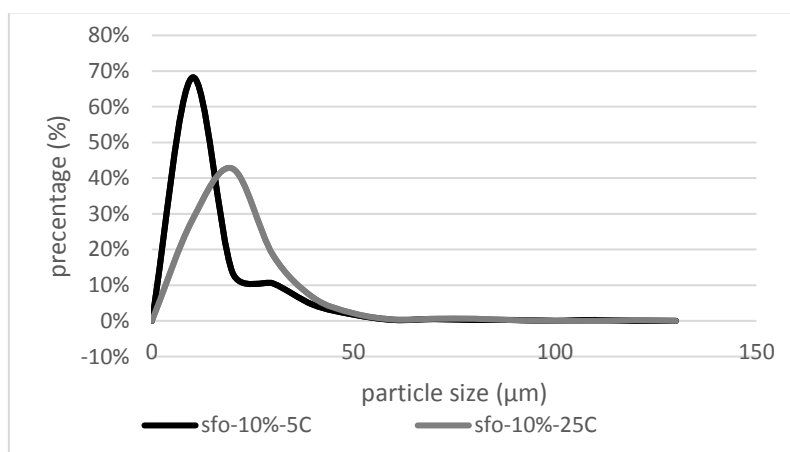
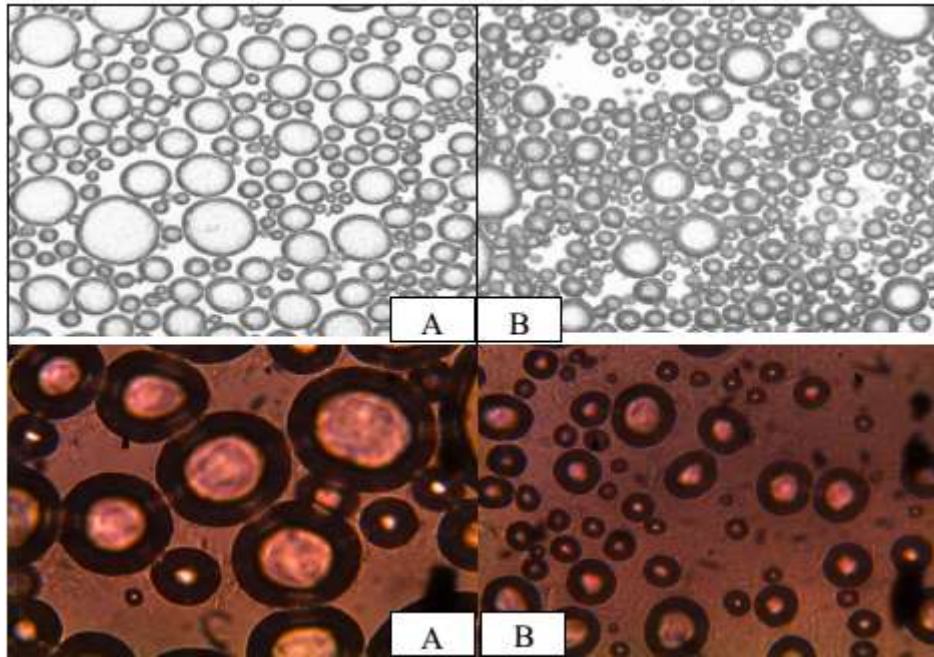


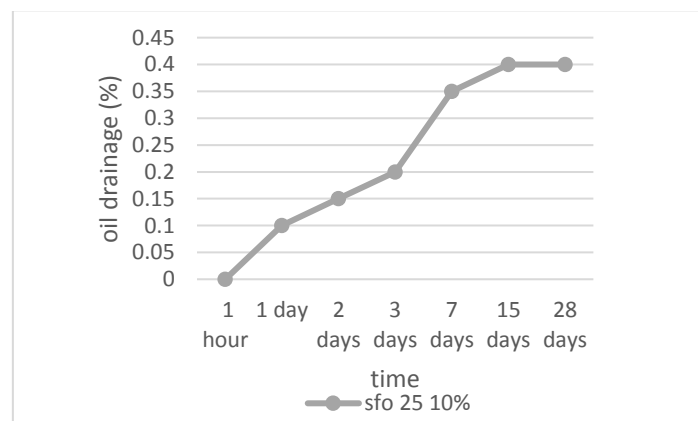
Fig. 2. Particle size distribution profiles of the 10% monoglyceride (MG) oleofoam



**Fig. 3.** Optical micrograph about the effect of temperature on particle size in sunflower oleofoams (a) at 25°C (b) at 5°C scale bars = 60μm (upper pictures) and (lower); polarized optical microscope image (a) at 25°C (b) at 5°C scale bars = 5μm

Small bubbles can be perfectly surfaced with a dense layer of MG crystals to protect them from coalescence and Oswald ripening (Callau *et al.*, 2020). Additionally, low temperatures enhance the formation of small bubbles, and this finding is consistent with study of Callau *et al.* 2020, which showed that fast cooling can decrease the size of bubbles and increase their

stability against oil loss. Also, Binks *et al.* (2016) discovered that the formation of stable foams was restricted to lower temperatures (22 °C and 30 °C) when utilizing crystal dispersions. Conversely, when employing molecular solutions, no foam formation was achievable at elevated temperatures (35 °C and 40 °C).



**Fig. 4.** Oil loss of oleofoam produced at 25 C during the aging time

#### A/O/W double emulsion formation

Generally, the oleofoam sample prepared at low temperatures showed higher overrun value, smaller bubble size, and oil drainage resistance;

thus, according to these results, it was selected for preparing the A/O/W double emulsion for further studies.

### Air encapsulation efficiency after A/O/W double emulsion formation

The results shown in Fig. 5 indicate that in the emulsion system, the level of sodium caseinate and kappa carrageenan significantly affects the amount of air encapsulated in the emulsion. In contrast, the level of oleofoam was not effective in this system. However, encapsulated air bubbles in all emulsions ranged from 87.8 to 117.3%. In comparison with other studies, it was relatively higher due to the high amount of air (~140 %) entering the system from the oleofoam. The amount of encapsulated air increased with an increase in the level of sodium caseinate. For example, in 20% oleofoam concentration with the presence of 8.0% kappa carrageenan, the level of air encapsulation increased from approximately 105% to ~111%, and in 25% oleofoam

concentration, it increased from approximately 110% to ~117%. This is because the droplets of the external oil in the A/O/W emulsion were subsequently stabilized by using sodium caseinate as an emulsifying agent, and an increase in concentration resulted in an increase in the number of encapsulated globules (Goibier *et al.*, 2019). Generally, this natural surfactant covers the surface of oil droplets and prevents their coalescence in the continuous aqueous phase (Thanh Diep *et al.*, 2018). Lu and colleagues examined the effects of sodium caseinate and acetylated mung bean starch on encapsulating lutein. They reported that increasing the amount of sodium caseinate resulted in increased lutein in the microcapsules (Lu *et al.*, 2021). Goibier and colleagues used sodium caseinate (12%) as an emulsifier to form stable emulsions (Goibier *et al.*, 2019).

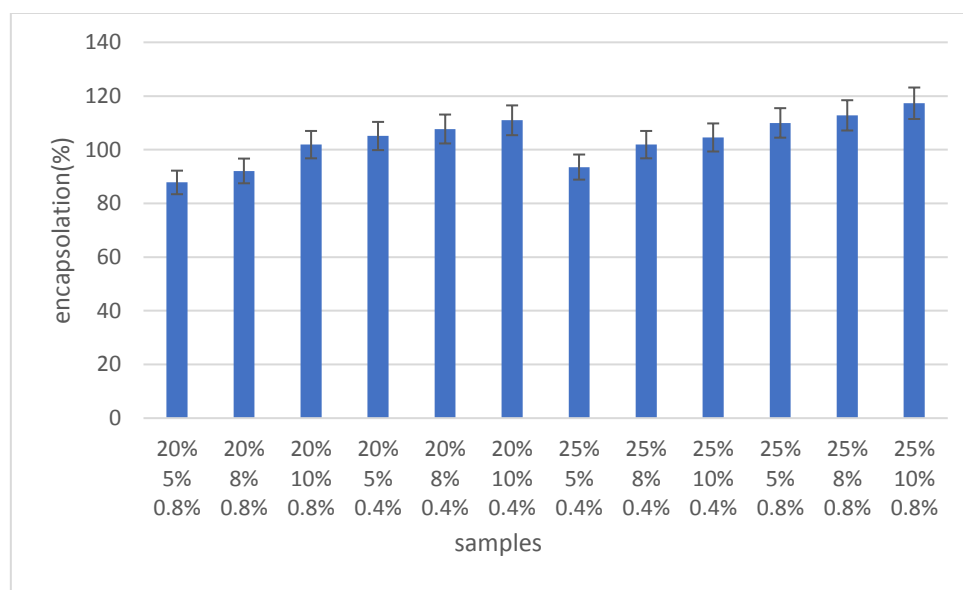


Fig. 5. Air encapsulation efficiency of (A/O/W) double emulsions

The results indicate that at different concentrations of kappa-carrageenan (0.4%, 0.8%), the amount of encapsulated particles containing air bubbles increases with an increase in sodium caseinate (i.e., it has a synergistic effect) because of the protein-polysaccharide interaction, which formed a more bulky polymeric layer at the interface. Furthermore, Kappa-carrageenan as a

thickening agent can serve as a suitable supporting additive to limit the phenomenon of flocculation and coalescence since it can increase the viscosity and cause better steric stabilization. Similarly, O'Regan & Mulvihill (2010) reported the combined effect of introducing polysaccharides into W/O/W emulsions. Specifically, they incorporated vitamin B12 into W/O/W emulsions utilizing



gelatin to solidify the inner water phase, medium chain triglyceride oil as the oil phase, PGPR as the lipophilic emulsifier, and either sodium caseinate or sodium caseinate-maltodextrin conjugate as the hydrophilic emulsifier. Their investigation revealed that the encapsulation efficiency of the double emulsion stabilized with sodium caseinate-maltodextrin surpassed that of the sodium caseinate-stabilized emulsion.

### Particle size

The size of particles present in emulsions has a crucial role in their stability, accessibility, biological activity, and physical and sensory properties. Smaller particle sizes lead to more desirable properties. The study results

presented in Table 1, demonstrate that the percentage of sodium caseinates and kappa carrageenan significantly affects the size of oleofoam droplets in the A/O/W double emulsion. However, the percentage of oleofoam did not significantly affect the final particle size.

The largest particle size ( $56.659 \pm 4.20$ ) micrometers was observed in the emulsion with the lowest percentage of sodium caseinates and kappa carrageenan. The smallest particle size ( $1.73 \pm 0.1$ ) micrometers was observed in the sample containing 20% oleofoam, 10% sodium caseinates, and 0.8 % kappa carrageenan. This A/O/W double emulsion can be considered a fine multiple emulsion because the particle size is between (1-3  $\mu\text{m}$ ) (Aserin, 2007).

**Table 1- Effect of oleofoam, SC, and KC percentage on the droplet size of A/O/W double emulsion**

Samples	D 3,4( $\mu\text{m}$ )
sfo20%, SC 5%, KC 0.4%	50.904 $\pm$ 5.62a
sfo20%, SC 8%, KC 0.4%	35.434 $\pm$ 2.60b
sfo20%, SC 10 %, KC 0.4%	24.756 $\pm$ 2.30c
sfo20%, SC 5%, KC 0.8 %	12.705 $\pm$ 1.30d
sfo20%, SC 8%, KC 0.8%	6.606 $\pm$ 0.30e
sfo20%, SC 10%, KC 0.8%	1.729 $\pm$ 0.01f
sfo25%, SC5%, KC 0.4%	56.659 $\pm$ 4.20a
sfo25%, SC8%, KC 0.4%	33.940 $\pm$ 2.03b
sfo25%, SC10%, KC 0.4%	23.559 $\pm$ 0.66c
sfo25%, SC5%, KC 0.8%	12.586 $\pm$ 0.89d
sfo25%, SC8%, KC 0.8%	7.3703 $\pm$ 0.74e
sfo25%, SC 10%, KC 0.8%	1.886 $\pm$ 0.17f

<sup>a-f</sup> Different case letters indicate statistically significant ( $P < 0.05$ ) differences among values.

Data represent the mean of duplicate determinations  $\pm$  standard deviation.

The diameter of the particles significantly decreased with an increase in the percentage of sodium caseinates. For instance, in the sample containing 20% oleofoam and 0.4 % kappa carrageenan, the diameter of particles decreased with the increasing of sodium caseinates from  $50.904 \pm 5.62$  to  $24.756 \pm 2.03$  micrometers. This reduction in particle size distribution is attributed to the increased protective effect of the sodium caseinates film coating at the oil-water interface. This finding is consistent with the results reported by Lin *et al.* (2020); it was found that increasing the percentage of sodium caseinates from 10 to 12% in a water-in-oil-in-water double emulsion

containing polyether-modified siloxanes (PMS1) emulsifiers resulted in a decrease in droplet size from 12.2 to 7.7 micrometers. Similarly, Dwyer *et al.* (2013) observed that increasing the concentration of sodium caseinates from 0.25 to 3 % reduced the mean particle size of oil-in-water emulsion (from 1179 to 325 nm).

As seen in Table 1, an increase in the concentration of kappa carrageenan leads to a reduction in the size of oleofoam droplets in A/O/W double emulsion. Specifically, in an oleofoam containing 5% sodium caseinates, an increase in kappa-carrageenan concentration from 0.4 % to 0.8 % resulted in a significant

decrease in droplet size from  $50.904 \pm 5.62$  to  $12.705 \pm 1.30$  micrometers. This reduction in droplet size can be attributed to the increased viscosity of the solution and decreased interfacial tension of the emulsion with an increase in kappa-carrageenan concentration. Thanh Diep *et al.* (2018) reported that an appropriate combination of 0.5

% sodium caseinates (SC) and 1% kappa carrageenan (KC) were found to stabilize an oil-in-water (O/W) emulsion with a 4:6 ratio and decrease the particle size to  $2.089 \pm 0.332$  ( $\mu\text{m}$ ). The interaction between sodium caseinates and kappa carrageenan has a synergistic effect on reducing the droplet size of oleofoam globules in the emulsions due to electrostatic interactions between the negatively charged sulfate groups present in kappa carrageenan and the positively charged amine groups in the sodium caseinates molecule. This results in weak adsorption of polysaccharides onto protein-coated oil droplets, causing the polysaccharides to remain in the continuous phase and increase the viscosity of the emulsion. The competition between these two processes determines the stability of the emulsion. Similarly, Perrechil & Cunha, (2013) showed that an increase in the concentration of sodium caseinates and kappa carrageenan leads to a decrease in the average droplet size of the emulsion.

#### **A/O/W emulsion stability**

In order to replace animal fat in food formulations, an A/O/W double emulsion was created. However, it was crucial to consider the thermal stability of this emulsion due to the need for pasteurization of the blended ingredients. This pasteurization process plays a significant role in preserving the quality of the final product. No significant separation was observed at room temperature and 4 °C during

30 days of storage, indicating excellent stability at room and low temperatures.

The results showed in Figure 6 indicate that the percentage of oleofoam used in the preparation of oil-in-water-in-oil (A/O/W) emulsion may be the most essential factor in determining its stability. Specifically, the analysis showed that the foam percentage significantly affects emulsion stability, with samples containing higher percentages of oleofoam and lower levels of sodium caseinates and kappa carrageenan exhibiting the highest thermal instability. The observed thermal instability is attributed to the inadequate levels of sodium caseinates and kappa carrageenan, which lead to the accumulation of oleofoam droplets. The droplets containing air bubbles are irregularly shaped and relatively large. This irregular shape is inherent to the foam's firmness and, during heating, causes the oil droplets containing the air bubbles to coalesce rapidly due to insufficient coverage. The monoglyceride crystals at the oil-air interface have reached their melting point, causing the oil to separate from the system. However, with an increase in the levels of sodium caseinates and kappa carrageenan, the coverage of the air-containing oil droplets increases and becomes more robust as the size of the globules is smaller. This prevents the oil from being released outside the system. Similarly, Lei *et al.* (2019) reported on the effect of heating on the thermal stability of oleofoam, stating that when the temperature reaches 60°C, the entire oleofoam is converted to a liquid state due to the melting of monoglyceride crystals at the oil and air interface in the continuous phase. It should be noted that in oleofoam, where the only emulsifying agent is fat crystals, the degree of instability is much higher than the results presented in this study.

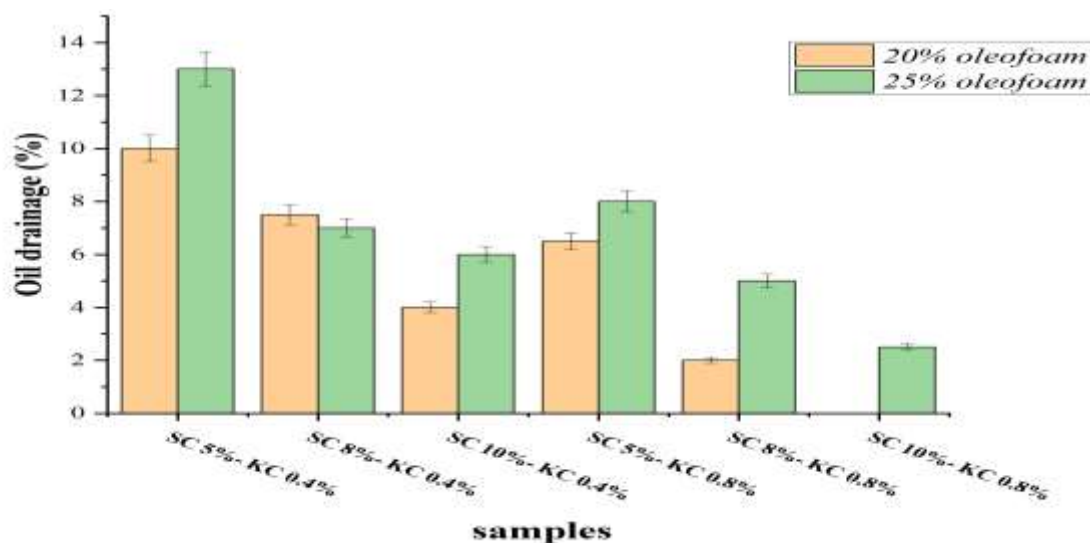


Fig. 6. Thermal stability (%) of A/O/W double emulsions

The percentage of sodium caseinate significantly affected thermal stability, as an increase in its percentage led to an increase in thermal stability. Increasing the percentage of sodium caseinate from 5% to 10% in samples containing 20% oleofoam and 0.8% kappa carrageenan increased stability and decreased oil separation from 6% to zero. The enhancement of stability was observed with an increase in sodium caseinate percentage, attributed to the reduction in size of air-containing oil droplets at higher concentrations of sodium caseinate. Additionally, a greater coverage of these oil droplets was evident at elevated levels of sodium caseinate. According to literature, the emulsions stabilized by caseinate tend to exhibit superior thermal stability due to the unique structure of this protein, which is resistant to conformational changes caused by heating. The combination of electrostatic and steric mechanisms of stabilization offered by sodium caseinate effectively protects emulsion droplets at neutral pH conditions (Sharma *et al.*, 2017). Li *et al.* (2017) examined the thermal stability of multiple emulsions containing different protein and polysaccharide combinations at 60 C. They found that adding protein compounds improved the stability of the emulsion, but the amount added was observed to be a more significant factor. As a result, increasing the percentage of

sodium caseinate from 0.2% to 1% increased the emulsion compounds' stability. These findings agree with the present study, indicating that the percentage of sodium caseinate plays a crucial role in improving the thermal stability.

Adding kappa-carrageenan increased the emulsion's stability, as emulsions with higher concentrations of kappa-carrageenan were found to be more stable because kappa-carrageenan increases the viscosity of the emulsion and forms small-sized globules. The results showed that with an increase in the percentage of kappa-carrageenan from 0.4% to 0.8%, the stability of the emulsion containing 20% oleofoam and 10% sodium caseinate increased from 95% to 100%. According to the report by Zhao *et al.* (2015), a three-dimensional network structure was formed mainly from carrageenan in the carrageenan-protein system. Similarly, Perrechil & Cunha (2013) reported the successful production of multiple emulsions using surface complexes of polysaccharide (kappa-carrageenan) and protein (sodium caseinate). They demonstrated that high concentrations of kappa-carrageenan led to the production of stable emulsions under neutral and acidic conditions. These findings suggest that kappa-carrageenan can effectively stabilize multiple emulsions.

Statistical results indicate a synergetic effect between kappa-carrageenan and sodium

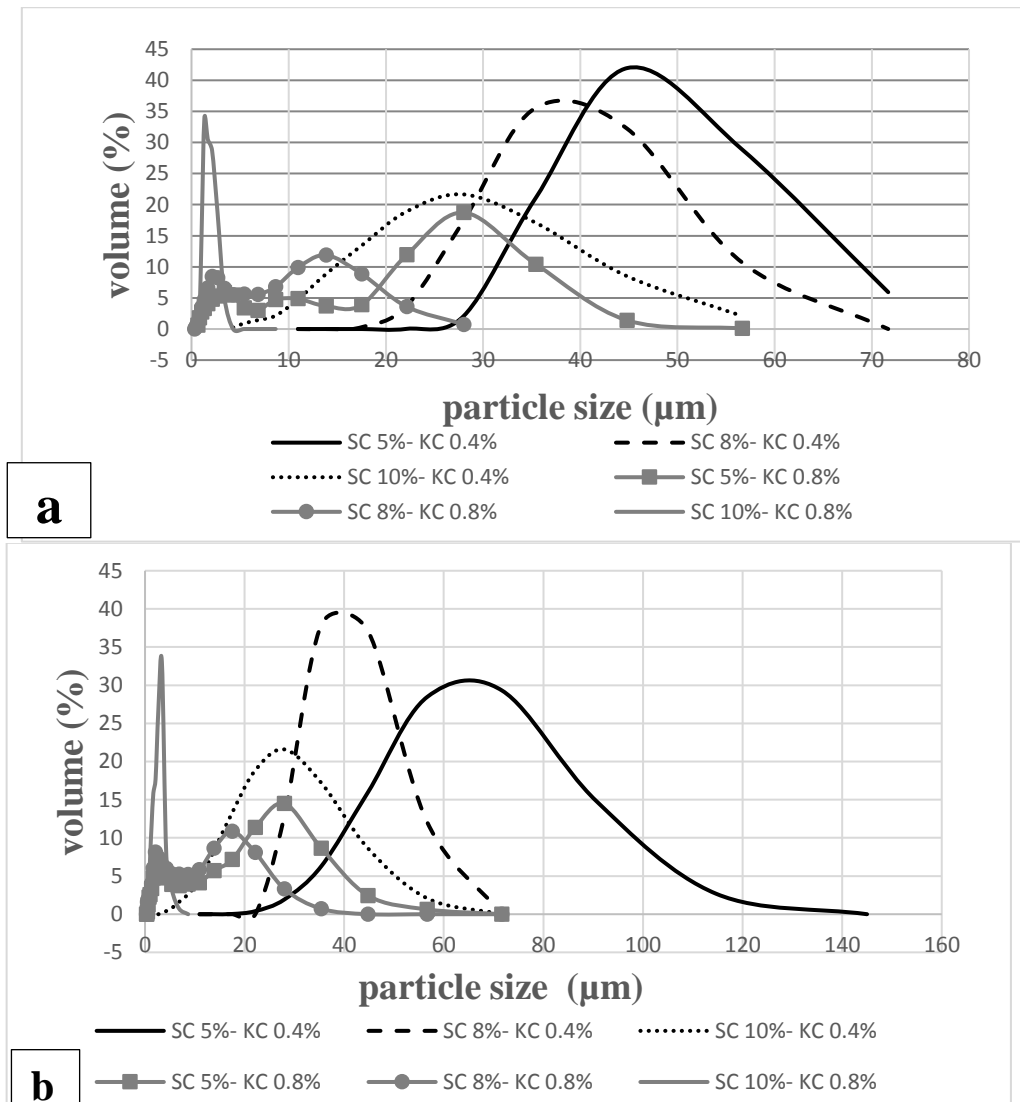
caseinate, whereby an increase in the concentration of both compounds positively impacts stability. This is due to the surface molecular interaction between casein and kappa-carrageenan, as reported by [Tang \*et al.\* \(2019\)](#), who found that adding kappa-carrageenan improved the thermal stability properties of casein.

### Optical microscopy

The particle size of a prepared A/O/W emulsion was examined to confirm the proper encapsulation of the oleofoam within the internal coating material. Microstructures of the samples were visualized immediately after emulsion fabrication for this purpose and shown in [Fig. 7a](#) and [b](#). The micrographs showed that large irregular particles were observed in the double emulsion with a lower percentage of sodium caseinate and kappa carrageenan ([Fig. 8a](#) and [b](#)) due to the intrinsic firmness of the oleofoam and low viscosity, which did not allow total shape relaxation. Conversely, with an increase the percentage of sodium caseinate and kappa carrageenan, smaller and uniformly size particles were observed in the double emulsion ([Fig. 8a](#) and [b](#)), indicating that this protein can act as a robust emulsifier at the interface of oil and water by reducing the interfacial tension. Moreover, sodium caseinate can generate electrostatic and steric repulsive forces between oil droplets, thereby hindering their proximity. According to Stokes' law, smaller oil droplets experience less resistance as they are more efficiently dispersed in the continuous phase, leading to slower coalescence and the formation of larger droplets or delayed flocculation ([Mollakhalili Meybodi \*et al.\*, 2014](#)). Sodium caseinate and kappa carrageenan can form a protective layer around the oil droplets, preventing them from coming into close contact and minimizing the potential

for aggregation. This steric stabilization is mainly attributed to the large size and complex structure of the protein, which creates a physical barrier between the droplets. Electrostatic regulation involves the repulsive forces generated between charged particles. Sodium caseinate, as a protein, carries a net negative charge due to the presence of ionic groups, such as carboxyl and phosphate groups. Kappa carrageenan, on the other hand, is a sulfated polysaccharide and also carries a negative charge. The negative charges on both sodium caseinate and kappa carrageenan molecules create electrostatic repulsion between the oil droplets, preventing their close proximity and reducing the likelihood of coalescence. Generally, smaller droplet size offers more interfacial surface area, which increases the emulsifier's absorption capacity and increases the emulsion stability.

In the current study, smaller oil droplet size ([Fig. 7a](#) and [8a](#)) was achieved using a relatively low concentration of emulsifier (sodium caseinate) and thickener (kappa carrageenan) compared to previous studies conducted by [Goibier \*et al.\* \(2019\)](#) and [Burn \*et al.\* \(2015\)](#). [Goibier \*et al.\* \(2019\)](#) utilized oleofoam-containing oil and dehydrated milk fat with an aeration rate of 30% and sodium caseinate (12%) as an emulsifier to prepare A/O/W emulsion. The emulsion was stabilized using a 10% hydroxyethyl cellulose solution. The droplet size obtained at the highest shear speed (7350/s) was approximately 10 micrometers. Similarly, [Burn \*et al.\* \(2015\)](#) prepared two A/O/W emulsions with 50 vol. % oleofoam, 5 wt% sodium caseinate (emulsifier), and 1 wt% hydroxyethyl cellulose (thickener). The average diameters of the air bubbles and oil droplets were 10 and 100 micrometer, respectively.



**Fig. 7.** Droplet size distribution of oil containing air in A/O/W double emulsion with a concentration of 20% foam(a), Particle size distribution of oil droplets containing air in A/O/W double emulsion with a concentration of 25% foam(b)

**Rheological properties**

Flow curves were obtained by an up-down-up steps program with the shear rate varying between 10 and 400 s<sup>-1</sup> (Fig. 9a and b). Flow curves exhibited different behaviors depending on the emulsion fractions (percentage of oleofoam, sodium caseinate, and kappa carrageenan).

The results obtained for the relation between viscosities of oleofoams and shear rates showed that the apparent viscosity of the A/O/W emulsion oil decreases with increasing shear rate. This behavior is consistent with the shear thinning property of emulsions, which is

usually observed in complex fluids such as colloidal suspensions. The emulsion had a high viscosity at low shear rates, indicating that the oil droplets were tightly packed together. However, with increasing shear rate, the droplets were forced to align and slide past each other, leading to a decrease in viscosity, which may be related to the deformation of oleofoam particles, destruction of air bubbles, and weak interactions between them under the influence of shear forces. This drop increases with increasing shear rate, indicating the dependence of viscosity on shear rate.



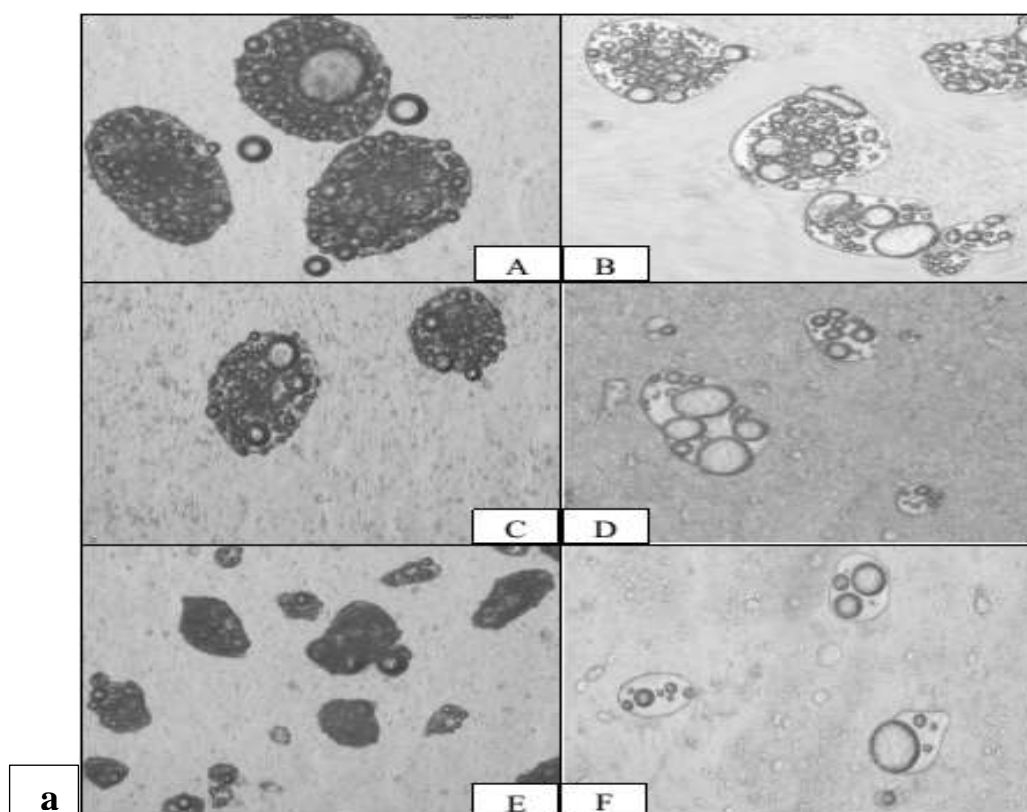
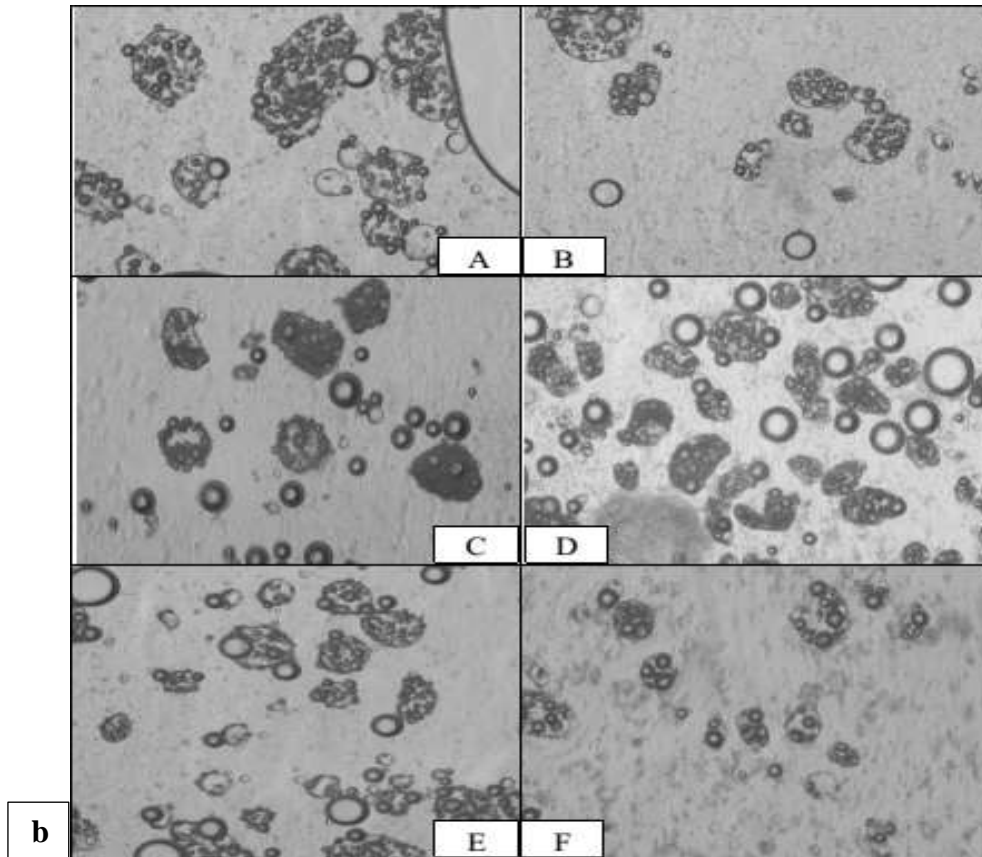


Fig. 8a. Microscopic images of oleofoams (a) oleofoam with 20% foam, 5% sodium caseinate, and 0.4% kappa carrageenan, (b) oleofoam with 25% foam, 5% sodium caseinate, and 0.4% kappa carrageenan, (c) oleofoam with 20% foam, 8% sodium caseinate, and 0.4% kappa carrageenan, (d) oleofoam with 25% foam, 8% sodium caseinate, and 0.4% kappa carrageenan, (e) oleofoam with 20% foam, 10% sodium caseinate, and 0.4% kappa carrageenan, (f) oleofoam with 25% foam, 10% sodium caseinate, and 0.4% kappa carrageenan (b)

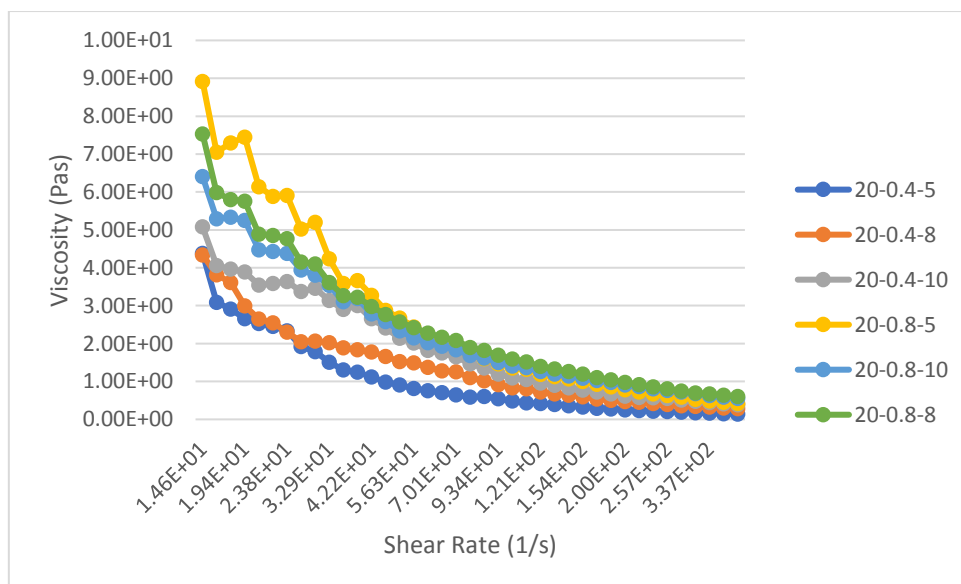
The flow curves showed the emulsions with high percentage of oleofoam (25%) were more viscous than those with 20%, which could be mostly related to high viscosity in oleofoam which was favorable for the gel-network formation and limited the movement of air bubbles (Fameau *et al.*, 2015). Increasing oleofoam fraction increases the monoglycerides percentage in the emulsion which traps oil droplets and increases resistance to shear. Liu *et al.* (2017), who investigated the effects of monoglycerides on the properties of protein-stabilized emulsions containing isolated whey protein and encapsulated  $\beta$ -carotene, reported that increasing the percentage of monoglycerides from zero to 2% led to an increase in emulsion viscosity, Davis *et al.* (2000) also reported for sodium caseinate-stabilized emulsions. Qiu *et al.* (2021) reported

that the viscoelasticity of the A/O/W emulsion was higher than that of the aqueous phase, indicating foam mixing in the emulsion slightly increased the stiffness of the mixture.

In general as seen in Fig. 9a and b, with an increasing percentage of kappa carrageenan, the viscosity of the samples increased in both oleofoams concentrations. The highest viscosity was observed in samples containing 0.8% kappa carrageenan; for example, in the oleofoam containing 20% and 5% sodium caseinate with 0.4% kappa carrageenan ( $5.38E+00$  Pas) was less than the sample containing 5% sodium caseinate with 0.8% kappa carrageenan ( $8.92E+00$  Pas), which could be caused by the higher concentration of free polysaccharides in the aqueous phase.



**Fig. 8b.** Microscopic images of oleofoams (A) oleofoam with 20% foam, 5% sodium caseinate, and 0.8% kappa carrageenan, (B) oleofoam with 25% foam, 5% sodium caseinate, and 0.8% kappa carrageenan, (C) oleofoam with 20% foam, 8 % sodium caseinate, and 0.8% kappa carrageenan, (D) oleofoam with 25% foam, 8% sodium caseinate, and 0.8% kappa carrageenan, (E) oleofoam with 20% foam, 10% sodium caseinate, and 0.8% kappa carrageenan, (F) oleofoam with 25% foam, 10% sodium caseinate, and 0.8% kappa carrageenan (b)



**Fig. 9a.** Flow curves of A/O/W double emulsion samples containing 20% oleofoam with different concentrations of sodium caseinate (5, 8, and 10%) and kappa carrageenan (0.4 and 0.8%)

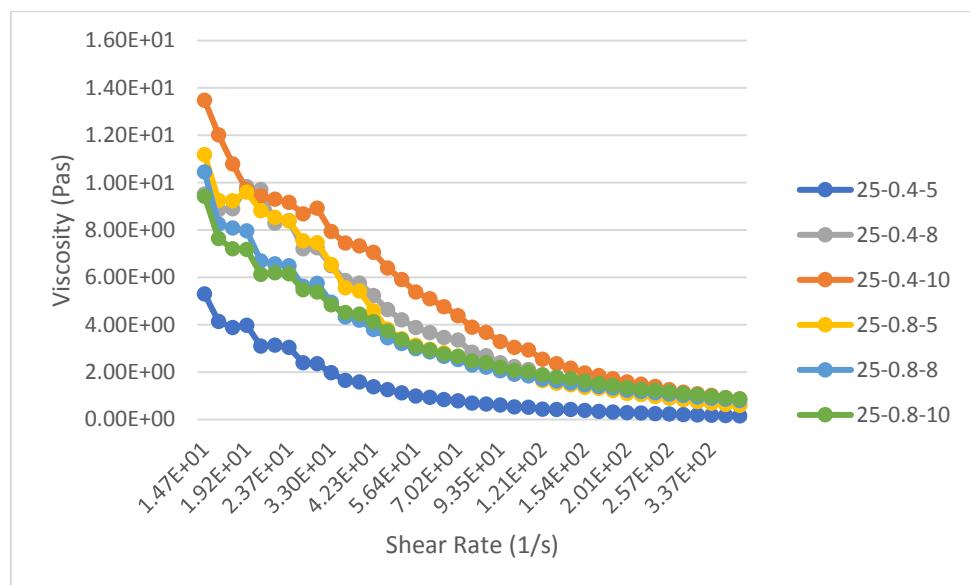


Fig. 9b. Flow curves of A/O/W double emulsion samples containing 25% oleofoam with different concentrations of sodium caseinate (5, 8, and 10%) and kappa carrageenan (0.4 and 0.8%)

These polysaccharide compounds have large hydrophilic groups and absorb much water, and increasing their percentage leads to an increase in the number of hydrophilic groups and more water absorption. In the presence of kappa carrageenan, the emulsion was significantly pseudoplastic, meaning that its apparent viscosity decreased with increasing shear rate and exhibited shear-thinning behavior.

Borsella *et al.* (2013) reported that increasing the percentage of kappa carrageenan at neutral pH in a multiple emulsion containing sodium caseinate led to an increase in viscosity. As seen in Fig. 9a and b, increasing the concentration of carrageenan has more significant effect on viscosity compared to the ratio of caseinate, and the highest viscosity is observed at higher concentrations of kappa carrageenan because sodium caseinate acts as an emulsifying agent and is responsible for the emulsification in this system. The same results reported by Perrechil *et al.* (2020) who investigated the effect of kappa carrageenan concentration on the rheological properties of beta-carotene encapsulation in multilayer emulsions containing SC at neutral and acidic pH.

Regarding the effect of sodium caseinate percentage on emulsion viscosity, the results

have shown that there have been two behaviors based on particle size and percentage. In samples containing 0.4% kappa carrageenan, increasing the sodium caseinate percentage increases the viscosity because the globule size is large in this concentration, and they require a low amount of sodium caseinate for coverage, while the rest of the sodium caseinate absorbs water and increases the viscosity. However, at higher concentrations of 0.8 % kappa carrageenan, the emulsion globule size decreases with increasing sodium caseinate percentage. Which sodium caseinate on the rheological behavior of different suspensions containing 20% oleofoam was smaller for the systems containing 25% oleofoam. This demonstrates the mutual effect between particle size and emulsion viscosity.

The fitting of rheological models reflected only the transient behavior of the samples and not their intrinsic properties, as Wei *et al.* (2018) reported. The power law model could fit the curves of oleofoams containing high percentages of sodium caseinate and kappa carrageenan, whereas the curves of other samples could not be fitted to any rheological model. This suggests that these samples exhibit unstable behavior and undergo changes in rheology at specific applied shear rates.

## Conclusion

This study aimed to evaluate the production of A/O/W double emulsion using a combination of proteins as emulsifiers and polysaccharides as thickeners with oleofoam. Initially, the oleofoam was prepared by incorporating air bubbles in a mixture of sunflower oil and MDG at two different production temperatures (5-25°C). The oleofoam prepared at 5°C exhibited higher overrun, smaller bubble size, and more stable foam than the one prepared at 25°C. Thus, it was selected for the preparation of A/O/W double emulsion. The results indicated that an increase in kappa carrageenan and sodium caseinate percentages increased the apparent

viscosity, promoted the formation of smaller oleofoam droplets with a narrow distribution, and increased the encapsulated particles containing air bubbles and thermal stability in the A/O/W double emulsion. These findings suggest that A/O/W double emulsion technology could enable the food industry to develop products with enhanced nutritional components, better appeal, and longer shelf-life, they pave the way for developing innovative food products benefiting both the food industry and consumers alike.

## Acknowledgements

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## مقاله پژوهشی

جلد ۱۹، شماره ۶، بهمن-اسفند، ۱۴۰۲، ص. ۱۹۵-۱۷۷

# بهینه‌سازی نسبت اولئوفوم و پروتئین-پلی ساکارید برای بهبود ویژگی‌های فیزیکوشیمیایی امولسیون دوگانه A/O/W: کاربردهای بالقوه در صنایع غذایی

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تاریخ پذیرش: ۱۴۰۲/۰۹/۲۰

## چکیده

تهیه امولسیون دوگانه هوا در روغن در آب (A/O/W) شامل دو مرحله کلیدی است: تشکیل اولئوفوم و پراکندگی اولئوفوم در محلول آبی حاوی پروتئین به عنوان امولسیفایر و هیدروکلوئید به عنوان عامل غلیظ‌کننده. این مطالعه با هدف بررسی اثر سطح اولئوفوم و غلظت‌های مختلف نسبت پروتئین-پلی ساکارید بر پایداری حرارتی، بازده کپسولاسیون و خواص رئولوژیکی امولسیون دوگانه A/O/W انجام شد. یک اولئوفوم با استفاده از یک امولسیفایر چربی‌دوست مونوگلیسرید مقطر (MG) و روغن آفتابگردان در دمای ۵ درجه سانتی‌گراد با حداکثر پایداری به دست آمد. دو سطح اولئوفوم (۲۰ درصد و ۲۵ درصد وزنی) به محلول آبی حاوی غلظت‌های مختلف کازئینات سدیم (SC) ۵، ۸ و ۱۰ درصد وزنی و کاپا کاراگینان (KC) ۰/۴ و ۰/۸ درصد وزنی اضافه شد. نتایج نشان می‌دهد که سطح اولئوفوم به‌طور قابل توجهی بر راندمان کپسولاسیون هوا و اندازه ذرات تأثیر نمی‌گذارد، درحالی‌که نسبت پروتئین-پلی ساکارید می‌تواند به‌طور قابل توجهی بر تمام خواص امولسیون دوگانه A/O/W تأثیر بگذارد. افزایش غلظت کازئینات سدیم و کاپا کاراگینان باعث بهبود پایداری حرارتی و راندمان کپسوله‌سازی شد درحالی‌که به‌طور همزمان اندازه ذرات را کاهش داد. همه امولسیون‌های A/O/W رفتار نازک شدن برشی را در میان طیف نرخ‌های برشی مورد مطالعه نشان دادند که نشان‌دهنده پتانسیل قابل توجهی برای کاربردهای غذایی است.

**واژه‌های کلیدی:** امولسیون دوگانه، اولئوفوم، نسبت پروتئین-پلی ساکارید، ویژگی‌های رئولوژی

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