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Quality and Stability of Emulsions Made of Whey Protein, Soy Protein, Arabic Gum, and Maltodextrin

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ABSTRACT

Introduction: Food safety and preservation methods are important issues, and food scientists and technologists are investigating new methods such as edible coating and microencapsulation. Most of these methods depend on the production of stable emulsions. The present study aimed to evaluate the effects of homogenizer speed, the ratio of the dispersed to the continuous phase, and the type of biopolymer on characteristics of emulsions.

Methods: In this study, Arabic gum (AG), soy protein concentrate (SPC), whey protein concentrate (WPC), and maltodextrin (DM) were used as biopolymers. Samples were divided into two groups based on the homogenizer speed and ratio of the dispersed to the continuous phase, including group one (14,000 rpm, 10% v/v) and group two (18,000 rpm, 20% v/v).

Results: On the first and sixth day of production, the smallest droplet size belonged to the samples produced by AG+DM in group one and those produced by SPC+DM in group two, respectively. The highest viscosity was observed in the samples of group two, which were produced by SPC+DM, while the lowest measured creaming index belonged to the samples in group two, which were produced by AG+DM on the first day of production. Finally, the most intense color based on the 'a' parameter was observed in the samples of group one, which were produced by AG+DM on the first day.

Conclusion: According to the results, the most stable emulsions could be produced by SPC+DM at 18,000 rpm.

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Introduction

Edible films and coatings are biodegradable compounds, which could inhibit the exchange of oxygen, carbon dioxide, and off flavor, thereby extending the shelf life of food [1]. In technologies, microencapsulation is considered an effective method to preserve sensitive materials by limiting environmental conditions and managing the controlled release of vital compounds (e.g., probiotics) in the human body, and the production of stable emulsions could be considered the first step in this regard.

Emulsion is a mixture of polar and non-polar liquids, which do not combine with each other. Therefore, it is necessary to use stabilizing materials, forces, and external pressures to produce emulsions [2]. Mechanical instruments such as homogenizers are often used in order to break particles into small quantities so that they could be spread throughout the emulsion bed. Moreover, using materials with high surface activity, thickening, and emulsifying properties could increase the stability period of emulsions [2, 3]. In brief, the stability of emulsions significantly depends on the particle size, type, and properties of the emulsifier, ratio of the dispersed phase to the continuous phase, and viscosity of the emulsion [4].

In most emulsions, the continuous phase is partly larger, and the dispersed phase droplets are distributed within the continuous phase [5]. In a stable emulsion, the excessive increase of the dispersed phase concentration may enlarge the droplets of the emulsion. Under such circumstances, more energy is required to break down the droplets, and the optimum ratio of the continuous and dispersed phase concentration should reach the maximum stability of the emulsion [6].

The materials that could be used in the continuous phase of emulsions as the stabilizer and emulsifier have wide categories and are

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mostly carbohydrates or protein-based substances [7]. Maltodextrin (DM) is a carbohydrate derived from the partial hydrolysis of starch. It is low-cost and has low viscosity in high solids content, a neutral taste, and acceptable ability to form films and stabilize emulsions [8]. Several studies have been focused on using this substance as a thickening agent and stabilizer in emulsions, demonstrating that using DM alone or combined with other ingredients within the range of 7-30% w/v could significantly extend the stability of emulsions [9, 10].

Arabic gum (AG), also known as the gum of *Acacia*, is extracted from wild trees mainly in the desert areas of Côte d'Ivoire, South Africa, Senegal, and Somalia. AG is made of glycoproteins and polysaccharides and is an abundant source of arabinose and ribose. AG has low viscosity even at high concentrations [11]. According to the literature, using AG as a stabilizer within the range of 2-8% w/v could increase the stability of the final emulsions during storage [12, 13].

Whey protein is a byproduct of the dairy industry and contains the soluble proteins of milk, which have a high nutritional value and considerable functional properties. Whey protein could be used as an emulsifier and a stabilizer in emulsions, gels, and foams [14]. Due to its highly nutritional and functional properties, this compound has been extensively investigated. Accordingly, adding whey protein concentrate (WPI; 0.5-4% w/v) and fixing the pH and other processing conditions could increase the stability of the final emulsion [15-17].

Soy protein is mainly available as an isolated soy protein (more than 90% protein known as SPI) and in the concentrated form (about 70% protein known as SPC). The main application of the isolate form is in the meat industry as a filler, thickening agent, water bounder, and emulsifier, while the concentrate form is often used in bakery, cereal, and meat industries. Both the isolate and concentrate forms of soy protein have notable functional properties as an emulsifier and stabilizer [18]. Researchers have reported that by adding soy protein concentrate to the formula of emulsions within the range of 0.5-3% w/v, the final products could have higher stability [19-21].

Given the importance of producing stable emulsions, extensive research has been focused on stabilizers, as well as the impact of various processing methods. Biopolymers are materials with favorable nutritional and functional properties. To date, several studies have investigated the effects of added whey protein isolate [15-17], soy protein concentrate [19-21], and AG [12, 13] for stabilizing emulsions. According to the obtained results, these biopolymers could extend the stability of emulsions individually or in combination with other compounds. However, no studies have compared these three common biopolymers or investigated the effects of processing conditions on the final products.

The present study aimed to evaluate the effects of the homogenizer power, type of biopolymer, and ratio of the dispersed to the continuous phase on the stability index of emulsions, such as the droplet size, creaming index, and color changes.

Materials and Methods

Lycopene, maltodextrin (DE20), 80% SPC, and 80% WPC were purchased from Anhui Minmetals Development Company (Anhuai, China), Qinhuangdao Lihua Starch Co. Ltd. (Qinhuangdao, China), Wachsen Company (Shandung, China), and Sapoto Cheese (USA), respectively. Soy oil was also provided from Salimi Industrials (Tabriz, Iran). Sodium azide and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (USA) and Merck (Germany), respectively.

Preparation of Emulsions

Maltodextrin was hydrated and dissolved with distilled water (25°C) overnight to ensure complete rehydration [22]. In the case of WPC, pH was adjusted to 8.0 using phosphate buffer, and the solution was heated at the temperature of 75°C for 30 minutes in a water bath (WB 14, Memmert, Germany) in order to denature proteins completely [23, 24]. Following that, maltodextrin was added with the ratio of 1:4.

The soy protein isolate was dissolved in distilled water, pH was adjusted to 8.0 using phosphate buffer, and the solution was heated at the temperature of 90°C for 30 minutes in a water bath in order to denature proteins completely [25, 26]. Following that, maltodextrin was added with the ratio of 1:9. AG was also hydrated with distilled water (25°C) overnight [27, 11], and the solution was mixed with maltodextrin with the ratio of 1:3.

Lycopene was dissolved in soy oil (5%w/v) and used as the dispersed phase of the emulsions. It was gradually mixed with solid material solutions (40% w/v) as the continuous phase using a rotor-stator homogenizer (D 91126, Heidolph Industries, Germany) at the quantities of 10% and 20% v/v.

The solid materials were prepared in three different formulas, including WPC+DM, SPC+DM, and AG+DM. To homogenize the emulsions, 10,000 rpm was initially used for five minutes, and the samples were completely mixed at 14,000 and 18,000 rpm for 10 minutes. In brief, the emulsions were divided into two groups based on the variables of emulsion processing (Table 1). At the next stage, the prepared samples were kept steady for six days, and changes in the droplet size, viscosity, color, and creaming index were monitored every day to investigate the kinetics of change.

Table 1. Groups of prepared emulsions according to independent variables

	Solid materials (% w/v)	Oily lycopene (%v/v)	Homogenizer speed (rpm)
Group 1	40	10	14000
Group 2	40	20	18000

Properties of the Emulsions

Droplet Size

The droplet size of the emulsions was measured using a digital microscope (VIVA-BW1008, Guangdong, China), which could be connected to a computer, and microscopic images of the samples were obtained. Following that, the droplet images were analyzed by the ImageJ software (1.44P). To capture the images, one droplet of the original emulsion was initially diluted in 100 milliliters of 0.1% w/v SDS [18], and one droplet of the diluted solution was placed on a laboratory lam and photographed using a microscope at the magnitude of 100x [28].

Viscosity

The viscosity of the emulsions was measured using a viscometer (D 220, Brookfield, USA) at the constant temperature of 30°C [28].

Creaming Index

In order to investigate the creaming index, 15 milliliters of the samples was stored in test tubes at the temperature of 30°C, and changes were monitored for one week [28]. The stability of the emulsions was characterized based on the creaming index using Equation 1, as follows:

$$Creaming\ Index\ (\%) = \frac{height\ of\ serum\ layer + height\ of\ cream\ layer}{total\ height} \times 100 \quad (1$$

Color

The image processing method was used to examine the color of the samples. For this purpose, a special box was used to control the light and capture accurate images in similar environmental conditions, and the images were analyzed by the ImageJ software [29].

Statistical Analysis

Data analysis was performed in SAS version 8.3 using the analysis of variance (ANOVA) and Duncan's test. All the experiments were carried out in triplicate, and the observed differences were considered significant at the P-value of less than 0.05.

Table 2. Measured specifications of emulsions at first day of storage

Specification	WPC		SPC		AG	
	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1
Average Droplet size	124.421^{d}	171.012^{b}	178.738^{b}	158.088^{c}	204.5^{a}	39.713^{e}
Viscosity	60.2^{d}	40.5^{e}	190.4^{a}	58.8^{d}	132^{b}	92.2^{c}
Color	4.518^{c}	2.825^{d}	4.202^{c}	1.756^{e}	5.799^{b}	6.015^{a}
Creaming index	2.9^{d}	16.16^{b}	0^{e}	63.26^{a}	4.78^{c}	4.71^{c}

Letters (a-e) in each row show significant difference in 5% level

Table 3. Measured specifications of emulsions at 6th day of storage

specification	WPC		SPC		AG	
	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1
Average Droplet size	243.39^{a}	222.224^{c}	196.849^{e}	208.278^{d}	231.649^{b}	198.64^{e}
Viscosity	52.4^{d}	35.1^{f}	153.7^{a}	45.8^{e}	106.5^{b}	74.2^{c}
Color	-3.663^{b}	-5.936^{d}	-4.506^{c}	-8.094^{e}	-3.25^{b}	1.3^{a}
Creaming index	12.78^{c}	18.34^{b}	11.89^{d}	79.23^{a}	11.02^{d}	11.84^{d}

Letters (a-f) in each row show significant difference in 5% level

Results

Kinetics of Droplet Size Changes

As is shown in Table 2, the samples were prepared in two groups, which differed in terms of the quantity of the dispersed phase (oily lycopene) and the speed of halogenation (rpm). Kinetics of the changes in the droplet size, color, and viscosity of the emulsions are presented in Figure 1 and Tables 2 and 3.

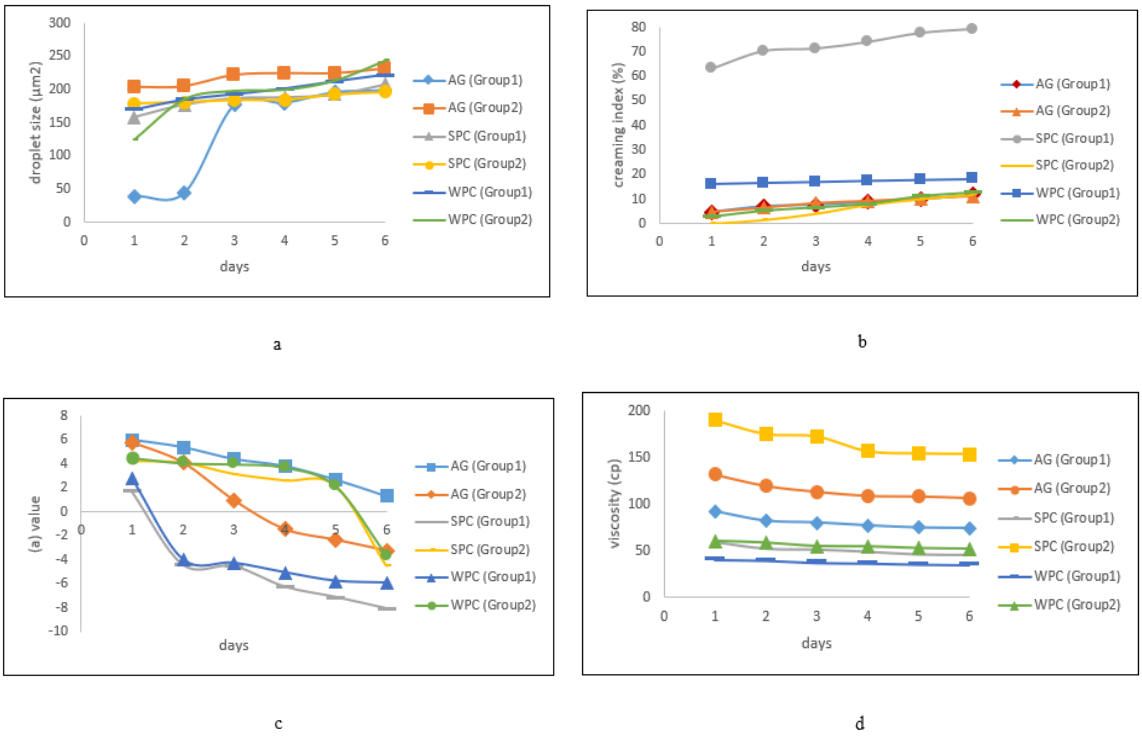

Figure 1. Kinetic of changes in emulsions, a: droplet size; b: creaming index; c: (a) value; d: viscosity

As is observed in Figure 1 and Tables 2 and 3, the droplet size and creaming index of the samples increased during six days of storage, while the viscosity and 'a' parameter of color decreased gradually. The AG samples in group one had the smallest droplet size, while the AG samples in group two had the largest droplet size on the first day. On the sixth day, the smallest and largest droplets sizes belonged to SPC in group two and WPC in group two, respectively.

As for the creaming index, the highest and lowest measured values were on the first day and belonged to SPC in groups one and two, respectively. On the sixth day, SPC in group one had the highest measured creaming index, while the lowest value was observed in the AG samples of group two at this stage. In addition, the highest viscosity on the first day was observed in SPC in group two, and the lowest value belonged to WPC in group one. After six days, the same results were obtained in all the samples. The AG in group one had the highest 'a' parameter according to the colorimetric analysis on the first day, while the lowest value belonged to SPC in group one. After six days of storage, the same results were obtained.

Discussion

Droplet Size

The droplet size of emulsions could significantly affect the physicochemical properties of emulsions. Reports in this regard suggest that the stability period of emulsions could be prolonged through reducing gravity forces and the aggregation rate of small droplets [30, 31]. This phenomenon can be proved by the Stokes' law, which was introduced by George Gabriel Stokes in 1851 to measure drag forces for very small spherical particles in viscous systems and emulsions (Equation 2).

$$\upsilon = \frac{(\rho_p-\rho_f)}{18\mu} gR^2 \quad (2$$

In the equation above, υ (m/s) is the speed of the particles/droplets of dispersed phase movement (settling/scrolling rate), ρ_p shows dispersed phase density (kg/m^3), ρ_p is the density of the continuous phase (kg/m^3), g is the gravity force (m/s^2), R represents the droplet radius (m), and μ is the viscosity of the system (Ns/m^2).

According to Equation 2, one of the parameters that may directly affect υ is the droplet size (square of their radius). Therefore, it could be concluded that decreasing the droplet size of the dispersed phase in an emulsion may positively influence reducing the rate of phase separation due to separating and dispersing the droplets within the continuous phase.

Various treatments could affect the droplet size of an emulsion during production. Homogenizers are a commonly incorporated instrument in this regard, which could produce massive external forces in different ways (e.g., high-speed rotation and shear stress) to break down and distribute droplets in the continuous phase bed [7]. On the other hand, the ratio of the dispersed phase to the continuous phase may affect the droplet size of emulsions. According to the information in Table 2, the ratio of lycopene (dispersed phase) to solid materials (continuous phase) was 1:4 and 2:4 in groups one and two, respectively. As a result, the samples of the first group had a lower amount of the dispersed phase compared to the second group.

Increasing lycopene could increase the viscosity of the dispersed phase and have a negative effect on the homogenizer performance. When the ratio of the continuous phase increases, higher energy and forces are required to break the droplets. This could be achieved by increasing the power and time of homogenization [6]. Furthermore, the higher ratio of the dispersed phase in an emulsion may increase the number of the droplets, thereby leading to a higher risk of re-coalesce and rate of flocculation, as well as larger droplet sizes [5, 32]. On the other hand, increased viscosity is directly correlated with the wall shear stress of dispersed phase droplets, which is illustrated in Equation 3, as follows [33]:

$$\tau_w = \frac{0.003496 \quad \eta_c^{0.25}}{\rho_c^{0.75} V_c^{1.75}} \quad (3$$
$$\pi^{1.75} D_i^{3.75}$$

where $_w$*shear* is the force on the droplet wall (pa)ד, η_c shows the viscosity of the continuous phase (cp), c is the density of the continuous phase (kg/m^{-3}), V_c represents the volume flow rate of the continuous phase (m^3/h^{-1}), and D_i is the inner diameter of the membrane tube (mm).

According to Equation 3, the shear force applied onto the droplet wall increases by increasing the viscosity of the continuous phase, thereby resulting in smaller droplet sizes. On the other hand, increasing τ_w could affect the viscose drag force (F_D in Eq. 4), which may increase the detaching forces versus the holding forces of dispersed phase droplets and result in smaller droplet sizes [34].

$$F_D = \frac{3\pi\tau_w d_{dr}^2}{2} \quad (4$$

In Equation 4, F_D is the viscose drag force (N), $_w$*shear* shows the force on the droplet wall (pa)ד, and d_{dr} is the diameter of oil droplets (dispersed phase; µm).

As the shear force applied onto the droplet wall increases, the viscous drag force increases and breaks the droplets down to smaller sizes. On the other hand, the solid materials used in the present study were biopolymers, which may act as emulsifying and thickening agents. Emulsifiers are surfactants that could reduce the interfacial tension between droplets and control the rate of phase separation [30].

$$W = \gamma\Delta A \quad (5$$

In Equation 5, W represents the free surface energy, γshows the interfacial surface force, and A is the surface Area (Δ).

According to Equation 5, droplets with a larger surface area and higher interfacial surface forces have higher free surface energy, which plays a key role in moving droplets and their joining. As emulsifiers could reduce interfacial surface forces, their presence in higher percentages could decrease the rate of phase separation.

Creaming Index

The creaming index represents the stability of emulsions. Higher stability of emulsions and a lower rate of phase separation result in a lower creaming index [34]. Every parameter that affects emulsion stability could also change this index; such examples are the droplet size, ratio of the dispersed to the continuous phase, and viscosity [35]. Larger droplet sizes and the higher ratio of the dispersed phase accelerate phase separation and negatively affect emulsion stability, thereby leading to a higher creaming index. Conversely, increasing the viscosity of emulsions may have a positive effect on their stability due to reducing the rate of droplet

movement and leading to a lower creaming index [5, 36]. The results of the present study indicated that increasing the biopolymer content and higher homogenizer power led to the production of more stable emulsions, which is consistent with the findings of Hosseini et al. (2015). Accordingly, different biopolymer ratios, homogenization periods, and storage temperatures could affect the stability of emulsions [13].

Viscosity

The viscosity of an emulsion is an important characteristic that affects the quality and stability of emulsions. Viscosity changes due to parameters such as the speed of the homogenizer, amount of the dispersed phase, and the quality and quantity of solid materials and thickening agents [37]. As viscosity increases, the phase separation rate of emulsions may decrease. Notably, thick emulsions could disrupt the effect of the homogenizer, thereby resulting in an emulsion with large droplet sizes and efficient droplet distribution [38].

According to the literature, the higher speed and rpm of the homogenizer could result in smaller droplet sizes, while also increasing the number of the droplets that are distributed within the emulsion [39]. As was shown in Equation 5, smaller droplets lead to higher free surface energy, which could motivate the droplets to attach to each other, and these droplet links may increase the total viscosity of the emulsion [40, 41]. On the other hand, the dispersed phase volume, which is obtained by dividing the volume of the dispersed phase droplets by the total volume of the emulsion, could significantly affect the viscosity of emulsions [30]. The overall viscosity of an emulsion eventually increases by increasing the amount of the dispersed phase, the interaction between the droplets, increasing the hydrodynamic and colloidal interactions, and the effects of these forces and interactions on the fluid flow [41-43]. According to the results of the present study, the samples with a higher content of biopolymers, oil, and homogenizer speed had higher viscosity. This finding is in line with the results obtained by Nahak et al. (2021) [19], Hebishy et al. (2017) [17], and Salimi et al. (2017) [20].

Color

The color of emulsions depends on the absorption or scattering of light waves in the visible region of the electromagnetic spectrum. The amount of the light scattered by emulsions could determine their turbidity, cloudiness, and brightness, while the absorbed light generally indicates the color of emulsions (blue, green, and red). The absorption and scattering of light by an emulsion may be affected by parameters such as the droplet size, dispersed phase volume, and the presence of color compounds in the emulsion. Furthermore, the light source emitted into the emulsion could affect the observed color [44].

To explain the effect of homogenizer speed on the color of emulsions, it is necessary to refer to the results of the effect of this independent variable on the droplet size of the emulsion. Homogenizer speed could have a significant effect on the droplet size of emulsions, and the droplet size may affect the amount of the absorbed or scattered light. Therefore, changing the speed of the homogenizer could affect the color observed in the emulsion to some extent, and the emulsions in which most of the droplets are less than 100 nanometers appear almost transparent (exceptions are the emulsions containing biopolymers as the surfactant) [45]. This is because light waves are reflected only by the droplets that are at least a quarter of the wavelength. The visible light spectrum has wavelengths within the range of 390-750 nanometers. If the droplets size in the emulsion is less than 100 nanometers, light penetrates into the emulsion and passes through the emulsion without significant scattering, and the emulsion seems transparent [46].

In the emulsions with larger droplet sizes than 100 nanometers, increasing the droplet size leads to higher color intensity. This implies that larger droplets are more capable of absorbing light, and the emulsion color seems more intense [47]. According to the current research, the samples with higher levels of oily lycopene had a more intense red color. In addition, the nature of each biopolymer and final viscosity could affect the visual color of emulsions. These findings are consistent with the results obtained by Salimi et al. (2017) [20] and Nahak et al. (2021) [19].

Effects of Biopolymer Quality and Quantity

In addition to factors such as homogenizer speed and the ratio of the dispersed to the continuous phase, other factors could also affect the stability of an emulsion. Such an example is the type of the solid (biopolymer) used in the continuous phase as the stabilizer. According to the literature, adding whey protein isolate [15-17], soy protein

concentrate [19-21], AG [12, 13] could effectively extend the stability and shelf life of various emulsions, which is consistent with the results of the present study.

Conclusion

Emulsions are important colloid systems, which do not tend to be stable. To extend their stability, various parameters could be considered, including the production process and the nature and ratio of the incorporated materials. According to previous studies, breaking down the droplet size and distributing droplets within the continuous phase could have a positive effect on the stability of an emulsion although it is not the only influential factor in this regard. Other characteristics such as viscosity also play a key role in this phenomenon. Emulsions with higher viscosity tend to be more stable, while high viscosity and adhesive medium may have negative effects on the homogenizer performance. On the other hand, the ratio of the dispersed to the continuous phase could affect the stability of an emulsion, and a higher ratio is associated with more instability. Overall, none of these parameters could be discussed individually, and to investigate the stability of emulsions, all of these parameters should be considered. According to the results of this study, using soy protein concentrate and maltodextrin as thickening agents and homogenizing at 18,000 rpm increased the stability of the produced emulsions.

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Conflicts of Interest

None declared.

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