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Effect of different concentrations of sodium chloride and soy protein isolate with mono- and diglycerides/corn oil on physical properties and stability of w/o/w double emulsion

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Abstract. The successful fabrication of double emulsion is determined by the ingredients or the process. The aim of the study was to determine the effects of sodium chloride and soy protein isolate with addition of mono-and diglycerides/corn oil on physical and stability of emulsion. Model emulsions with different concentration of sodium chloride (0.5% and 1.5%) and soy protein isolate (1% and 3%) with addition of mono-and diglycerides/corn oil (ratio 1:1) were prepared. Physical properties and stability of double emulsion were characterized by viscosity, emulsion stability, pH, and color. The results showed that the stability of double emulsion was significantly affected ($p < 0.05$) by the interaction between sodium chloride and soy protein isolate, whereas pH and viscosity were affected only by the concentration of soy protein isolate. However, double emulsion containing 1.5% of sodium chloride and 3% of soy protein isolate (SPI) with addition of mono-and diglycerides/corn oil showed the highest stability in comparing with emulsion containing 0.5% of sodium chloride and 3% of SPI.

1. Introduction

An emulsion is a mixture of two or more liquids that are immiscible, in which one liquid is dispersed in the other [1]. Thus, two phases are present, one is continuous phase and another one is dispersed phase. Generally speaking, the two phases are oil phase and water phase. Emulsions can be divided into food manufacturing conventionally into two as water-in-oil emulsions (W/O) and oil-in-water (O/W). Multiple emulsions, mentioned as "double emulsions" or "emulsion of an emulsion" in literature [2], are multi-compartmentalized systems in which oil-in-water (O/W) and water-in-oil (W/O) at the same time, where the globules of the dispersed phase themselves contain even smaller dispersed droplets [3]. Different ways to simplify complex double emulsions exist: the reduction of phases, increase of the size of the droplets, thus reducing the curvature of the interfaces, and the observation of single droplets. The measurement methods stay the same: optical measurements, the measurement of droplet size, marker concentration, creaming rate, or rheological characteristic values (e.g., viscosity) [4]. The standard procedure to make a double emulsion involves 2 separate stages. The primary W1/O emulsion prepared using a lipophilic emulsifier is called the 1st stage while the 2nd stage provides W1/O/W2 emulsion using a different hydrophilic emulsifier. It has long been recognized that the choice of methodology in the secondary emulsification step (dispersing W1/O into W2) is critical for the preparation of a well-behaved formulation [5]. According to Vladisavljević et al., 2017, double emulsion can be prepared using various methods, such as high-pressure homogenizers, rotorstator homogenization, ultrasound emulsification, microfluidic emulsification and membrane emulsification [6]. Multiple emulsion has been suggested to advantages for use in food applications compared to single emulsion because its matrices can be used for the encapsulation of bioactive compounds but also for a controlled release of such compounds. Some authors reported that multiple emulsion is a good source of fat replacer in meat systems [7]. In addition, other authors also reported that multiple emulsions can be produced to influence sensory properties particularly taste perception by modifying the extent to which the aqueous phase interacts with oral surfaces [2]. Emulsion of this kind can be used for the production of foods with novel textures or low-fat products. The main problem during multiple emulsion fabrication were destabilization such as separation due to gravity (creaming and sedimentation), aggregation of droplet (flocculation and coalescence), and growth of droplets (Ostwald ripening) [8]. Stability of emulsion is very important because it affect the shelf life of food products. Researchers reported that emulsion destabilization is also caused by different physicochemical properties of each ingredient used in food product [9]. In addition, another problem in multiple emulsion production is to keep the stability of two different interfaces during processing and storage.

Development of multiple emulsion-based foods is one of strategies that exhibits potential to be used for enhancing saltiness and fat perception since their application on food affect sensory attributes. Jimenez-Colmenero reported the positive effect of multiple emulsion w/o/w on food such as improve fat content, encapsulate (protect) bioactive compounds, and reduce sodium content [10]. However, multiple emulsions are more difficult to be prepared in comparing with single emulsion due to existence of large droplet and tendency to release entrapped compound [3].

Emulsion systems in foods does not only contain oil, water, and emulsifier but also various additives such as hydrocolloids, proteins, especially salts in product formulations. The term “salt” is widely known as sodium chloride. It is used in food products since it gives taste and inhibits microbial growth [11]. For example, mayonnaise, ketchup, and sauces include approximately 0.6-1.2, 0.4-1.9, and 0.6-3.6 wt% sodium, respectively. The interaction between the emulsifier and salt could impart an important effect on the emulsion stability and rheology.

Soy proteins are amphiphilic molecules and usually used as emulsifiers in the food emulsion systems because of their structure and properties at the oil–water interface. Soy protein could exist at the oil–water interface and reduce the interfacial tension between the phases, which might provide steric and electrostatic repulsion between the oil droplets to form a stable emulsion [12][13].

Mono- and diglycerides (MDGs) are emulsifiers used to modify physical properties and creaming stability in protein-stabilized emulsion. Monoglycerides exhibit one hydrophobic fatty acid esterified to the hydrophilic glycerol molecule, while diglycerides exhibit two fatty acids. Mono- and diglycerides are oil-soluble molecules and exhibit very low solubility in water [14].

The aim of the present study was to determine the effects of sodium chloride and SPI with addition of mono-and diglycerides/corn oil on physical and stability of double emulsion. To reach this goal, four double emulsions, corresponding to a full factorial design based on two levels of sodium chloride concentration and two levels of SPI, were produced.

2. Materials and Methods

2.1. Materials

The ingredients used for the double emulsion were sodium chloride (NaCl) (Merck, Germany), soy protein isolate (SPI) (Crown Soya Protein, China), mono-and diglyceride (MDG) (Food Group Limited, China), corn oil, water, and potassium chloride (Merck, Germany). All the ingredients were food-grade and obtained from a local supermarket.

2.2. Methods

Preparation of double emulsion. Twelve double emulsions, varying in their concentration, were produced in the present study according to a full factorial design based on two factors: the sodium chloride level (0.5% and 1.5%) and the SPI level (1% and 3%). To produce double emulsion, A w/o emulsion was initially prepared with KCl solution. The oil phase contained ratio MDG: corn oil (1:1). One hundred-gram batches of this w/o emulsion were formed by adding both phases to a glass beaker followed by mixing with ultraturrax at 6500 rpm for two minutes. Then, the double emulsion was obtained by emulsifying 48 g w/o emulsion into aqueous phase containing SPI and NaCl. The double emulsions were mixed with ultraturrax at 6,500 rpm for 2 minutes.

2.3. Physical and Stability Measurements

Stability of emulsion was determined according to the emulsion formation ability after heating and centrifugation. The emulsion sample was heated at 80°C for 30 minutes and then centrifuged (Eppendorf 5810 R) at 1,300 rpm for 10 minutes. The volume of the mixture, which still forms the emulsion, was measured, and the stability of the emulsion is calculated based on the equation [15]:

$$\%S = \frac{volume\ of\ the\ emulsified\ mixture\ (ml)}{total\ volume\ of\ the\ mixture\ (ml)} \times 100\%$$

Viscosity measurements of emulsion were performed by Brookfield viscometer model RVT (VM-BF-RV-01) with spindle number 1 and 2. Measurement of emulsion's color was performed by Minolta CR-300 chromameter. The scale used was scale L* (brightness), a* (chromatic color red-green), and b* (blue-yellow chromatic color). Testing was done by putting sensors to surface of emulsion and firing rays on two different parts [16]. Measuring was done three times for each section. Then, the obtained data were averaged. The pH of double emulsions was tested at room temperature using a pH meter (Oakton pH 510 series).

Data analyses were performed using R software. ANOVA was used to determine significant differences among the mean of physical properties and stability of double emulsion values calculated. Three replications were used to determine physical properties and stability. For all data analyses, the effects were considered significant when $p < 0.05$.

3. Result and Discussion

3.1. Stability

Table 1 showed the summaries of mean stability of double emulsion varying in NaCl and SPI level. Result showed that varying NaCl and SPI level concentration on emulsion significantly affected stability. Indeed, the interaction between NaCl and SPI also significantly affected stability of emulsion (ANOVA; $F(1;8)=168.88$, $P < 0.0001$) (Table 2). Figure 1 showed that the use of NaCl and SPI at a higher concentration helped increase the emulsion stability both in the initial day and the fifth day period, even though on the fifth day, the emulsion stability decreased (Table 3). Compared to NaCl and SPI at a higher concentration, the results exhibited decreased stability as NaCl and SPI concentration were lower. Researchers suggested that this may be attributable to the collision of SPI at high NaCl levels. Similar trends in stability of emulsion were reported by Hong et al. (2012) [17] who reported that stability emulsion increased with increasing NaCl concentration.

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Table 1. Physical properties and stability of double emulsion (mean ± sd)

Treatment		Parameters					
NaCl (%)	SPI (%)	Stability (%)	pH	L	a	b	Viscosity (cp)
0.5	1	4.17±1.0	6.22±0.01	50.43±0.12	10.63±0.03	5.60±0.10	16.83±5.06
	3	27.08±1.0	6.26±0.01	18.33±15.10	18.33±15.13	18.57±15.17	29.23±8.36
1.5	1	5.21±0.8	6.23±0.02	18.23±14.65	18.36±14.59	18.27±14.69	15.10±6.10
	3	37.50±0.00	6.27±0.00	17.87±14.51	17.73±14.34	17.90±14.42	31.17±3.81

Table 2. ANOVA on physical properties and stability of double emulsion.

		Stability (%)	pH	L	a	b	Viscosity (cp)
NaCl level	F (1;8)	4224.38	0.93	1.65	0.40	0.22	0.0003
	p-value	<.0001	0.36	0.23	0.54	0.65	0.98
SPI level	F (1;8)	624.76	27.00	1.56	0.39	0.24	5.50
	p-value	<.0001	<.0001	0.24	0.54	0.63	0.04
NaCl*SPI	F (1;8)	168.88	0.04	1.52	0.46	0.27	0.09
	p-value	<.0001	0.85	0.25	0.51	0.61	0.77

Table 3. Comparison of % emulsion stability

Emulsion	Emulsion stability (%)	
	Day 0	Day 5
NaCl 0.5% SPI 1%	4.17±1.0	3.12±0.1
NaCl 0.5% SPI 3%	27.08±1.0	6.25±0.00
NaCl 1.5% SPI 1%	5.21±0.8	6.25±0.02
NaCl 1.5% SPI 3%	37.50 ± 0.0	15.62±0.03

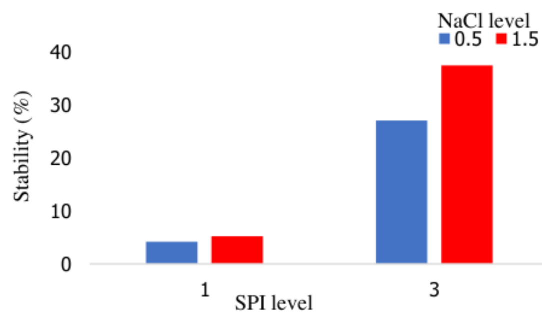

Figure 1. Influence of soy protein isolate (SPI) and sodium chloride (NaCl) on stability of emulsion

The most important properties which may contribute to the emulsion stability include pH. The results of the effects of NaCl, SPI, and their interaction on pH of emulsion were presented in Table 1. Table 2 showed that the NaCl level displayed no significant effect on pH (ANOVA; $F(1;8) = 0{,}93$, $p = 0.36$), whereas the addition of SPI showed a significant effect on pH (ANOVA; $F(1;8) = 27.00$, $P < 0.0001$). Emulsion made with the SPI level (3%) showed the highest pH in comparing with the SPI level 1% on both NaCl level. It is apparent that more added SPI can change the environmental condition including pH. SPI exhibits good gelation, emulsifying, foaming, and water absorption properties [18]. The interaction between NaCl and SPI also revealed no significant effect on pH ($F(1;8) = 0.04$, $P = 0.85$).

An emulsion-based delivery system may be incorporated into food products. Therefore, determining the color, such as lightness–darkness (L*), redness–greenness (a*), and yellowness–blueness (b*), is important. The results of the effects of NaCl, SPI and their interaction on color of double emulsions were presented in Table 1. Table 2 showed the concentration of NaCl, SPI, and their interaction revealed no significant effect on color ($p > 0.05$), which indicated that the color of emulsion was not different for all treatment, suggesting that an increase NaCl and SPI concentration resulted in no degradation of colour between the two aqueous phases and oil globule

The results of the effects of NaCl, SPI, and their interaction on viscosity of emulsion were presented in Table 1. The level concentration of NaCl and interaction between NaCl and SPI displayed no influence on viscosity ($p > 0.05$), whereas the SPI level was found to be significant on viscosity (ANOVA; $F(1;\ 8) = 5.50$, $p = 0.04$), which indicated that the viscosity between 1% and 3% were different. Figure 2 showed that a higher amount of SPI used (3%), the highest viscosity values obtained. It may be due to the presence of protein, which can absorb the surfaces of freshly formed oil droplets created by the homogenization of oil–water–protein mixtures [19].

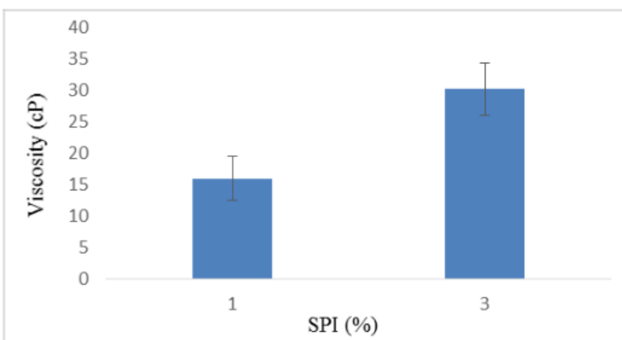

Figure 2. Influence of soy protein isolate on viscosity of emulsion

4. Conclusion

Based on the results of observations on emulsion, ingredients used such sodium chloride and SPI affects the physical and stability of double emulsion. Interaction between sodium chloride and soy protein isolate demonstrates a significant relationship with stability, and soy protein isolate alone demonstrates a significant relationship with pH and viscosity. However, the color of double emulsions were found to be no different. Therefore, further studies are necessary to determine the optimum level of NaCl and SPI.

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