

Properties of Emulsions Based In Soybean Oil Stabilized By Different Proteins

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Abstract

Different Soybean Oil-in-water emulsions (10 % to 50 % oil) were prepared at pH between 5.8 - 6 by using soybean protein isolate (SI - 4, 5 and 7 %) and casein (3 %). Emulsions prepared with high mass % soybean oil had a smaller average droplet size and exhibited greater stability. All emulsions prepared by soybean protein isolate exhibited higher stability than prepared with mix of SI + casein. In some samples where NaCl was added with different mass % (between 1 – 5), it was established that emulsion with addition of 2 % NaCl increases their stability. Gibbs free energy, enthalpy and entropy for each emulsion were calculated. One phase diagram for emulsion 5 was constructed. These diagram indicated phases which were determined experimentally.

Keywords: *Oil-in-water emulsions, Soybean oil, Protein stabilizers, Ternary phase diagrams.*

1. Introduction

Soybean oil/water emulsions combined with different proteins are appropriate to be used as emulsions for food industries [1]. Proteins are used as emulsifiers to facilitate the formation, improve the stability and provide specific physicochemical properties in emulsions [2]. More emulsions oil/water and water/oil were applied in meat industries in some meat products [3-5]. The development of protein-stabilized emulsions with improved physicochemical properties relies on understanding the interfacial behavior of adsorbed proteins, and the relationship between interfacial characteristics and bulk physicochemical properties of emulsions. Properties of emulsions stabilized by sodium caseinate-chitosan complex were investigated by Zinoviadou et al. [6]. Emulsions stabilized with milk proteins in recent developments by Dickinson and Yamamoto [7] and Dickinson [8]. The Faldt et al. [9] investigated soybean oil emulsions stabilized with whey protein and with lactose addition. Hsu et al. [10] prepared a soybean oil-in-water emulsion using non-ionic Tween series surfactants. The influence of pH and electrolyte on the ζ potential of emulsion drops had been examined and found that ζ

potential exhibit strongly dependent on pH. The McNiff et al. [11] investigated emulsion use of 10 % soybean oil. Lee et al. [12] used soybean oil as a biodegradable extracting agent for the removal of sorbet phenanthrene (PHE) in sandy soil. In their study, several methods of forming emulsions using soybean oil and their extraction properties has been investigated. Nikovska [13] prepared oil-in-water emulsions (o/w) with olive oil stabilized by soy protein isolate (SI) and whey protein isolate (WPI). It has been established, that the emulsions at lower oil concentration were less stable. The turbidity of emulsions was investigated by Ly et al. [14] and Mikkonen et al. [15]. They found that, the oil droplets of the emulsion moved upwards due to gravity which led to the formation of a relatively clear serum layer at the bottom of the cuvette. The turbidity of the emulsion in the bottom part of the cuvette indicates the stability of emulsion. Roesch [16] and Gancz [17] have investigated emulsions prepared with soybean protein and whey protein – as a stabilizer of systems. The objective of this work is to investigate emulsion stability with different soybean oil amounts and at two additions of protein: soybean protein isolate and casein.

2. Experimental

2.1 Chemicals

The soybean oil was used for all emulsion preparation. A stabilizer was used in soybean protein isolates (SI) 90 % (90 % total content) and casein (100 % pure protein). The emulsions had been prepared as follows: the SI 4, 5 and 7 % were added in water and soluble in it (% water indicates in Table 1). After that soybean oil was slowly added (Table 1). The emulsions were prepared with homogenizer with 2 minutes and 30 sec of mixing.

2.2 Determination of pH

pH in samples have been determined by pH meter Hanna HI 98127 with Replaceable Electrode.

Citric acid was used for correction of pH values of emulsions in continued phases. All emulsions were prepared in pH interval between 5.8 – 6.

2.3 Microscopic observation

The emulsions have been investigated with digital microscope Bresser. They were observed 10 minutes after their preparation. Digital microscopes, like the Bresser Junior USB Hand-held Microscope, are powered by the USB port on a PC. The images from the MikroCam MP Microscope camera have been prepared for all samples. According to the experimental results the particle size has been measured in micrometers.

2.4 Determination of emulsion turbidity

The investigations were performed with Camspec M550 Double Beam Scanning UV/VIS spectrophotometer. The emulsion was placed into a 1 cm plastic spectrophotometer cuvette Roesch et al. [17]. The change in the emulsion turbidity was measured at 350 nm 10 minutes after preparation of emulsion samples.

The system soybean oil/water/soybean protein isolate (SI+casein) has been investigated for 16 emulsion samples. Table 1 shows the chemical composition of emulsion preparation.

3. Results and discussion

To determine emulsion stabilities, turbidity of all samples has been measured. Investigation of samples have been prepared at 350 nm, and the middle radius of emulsion droplets has been calculated. The turbidity was measured 10 minutes after preparation of each emulsion. When the emulsion has small droplets it has high stability. When the emulsion has big droplets it has low stability. The more mass % of oil increases, the more the emulsion stability improves and thus the droplets have smaller size. The results of the calculated middle radius are presented in Table 1. After that optical microscope observations have been used again to determine droplet size. The pictures show that with the increasing of mass % of oil and the mass % of protein, smaller droplets appear. This indicates that the emulsions exhibit more stability.

Fig. 1 presents microscope pictures of samples 1 and 4.

Table 1. Chemical composition of emulsion samples 1-16 used to investigated system O/W/Protein

N ^o	Soybean oil, %	Water, %	Protein, %	pH	r, μm
			SI		
1	20	76	4	5.98	35.72

2	30	66	4	5.97	30.22
3	40	56	4	5.96	24.73
4	50	46	4	5.96	16.58
5	20	75	5	6.00	31.98
6	30	65	5	5.98	29.23
7	50	45	5	5.98	35.72
8	20	76	5	5.97	30.22
9	20	73	7	6.00	28.46
10	30	63	7	5.95	21.98
11	40	53	7	5.83	19.23
12	50	43	7	6.00	16.49
			SI+Casein		
13	20	77	1.5+1.5	6.11	43.96
14	30	67	1.5+1.5	6.05	32.97
15	40	57	1.5+1.5	5.82	30.22
16	50	47	1.5+1.5	5.80	27.46

The chemical compositions are shown in Table 1. Visible in the figure of sample 1, are bigger droplets and of sample 4, smaller droplets with increased mass % of oil.

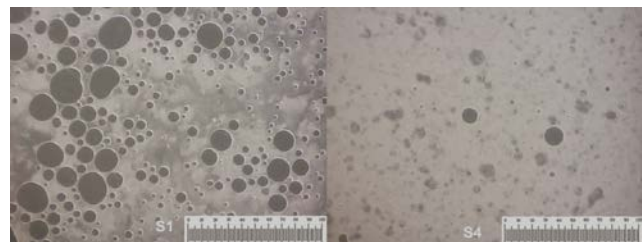


Fig. 1. Microscope pictures of sample 1 and sample 4, 10 minutes after preparation of emulsions.

Fig. 2 presents micrograph of optical microscope of sample 5, 10 minutes after preparation of emulsion (2c) and emulsion after 24 hours of preparation (Fig. 2 a, b). Three phases are visible (Fig. 2d): water, emulsion w/o (water/oil) and emulsion o/w (oil/water). Fig. 2 a, b presents two phases w/o and o/w determined as staining solubility test. Water soluble Xsilenolorange solution of 1 ml has been added to the emulsion phase. If the continuous phase is water (o/w emulsion), the indicator is dissolved uniformly throughout the system. If the continuous phase is oil (w/o emulsion), the indicator remains as a cluster on the surface of the system Dickinson [18], Syed [19]. Four phases were determined after investigation of all emulsions. In the samples with 20 % to 30 % oil and 4 % SI, three phases have been observed: water phase, emulsions o/w and w/o. In the samples with 20 – 40 % oil and 5 - 7 % SI, it has been observed in emulsions o/w and w/o. In the samples with 50 % oil and 4 % SI, it has been observed in emulsions w/o and some areas with gel structures. In the samples with 50 % oil and 5 % SI, 40 % - 50 % oil and 7 % SI, the sample is entirely

gel structure [11]. After experimentation, emulsion 5 exhibited 28 % water phase, 40 % o/w and 32 % w/o emulsions.

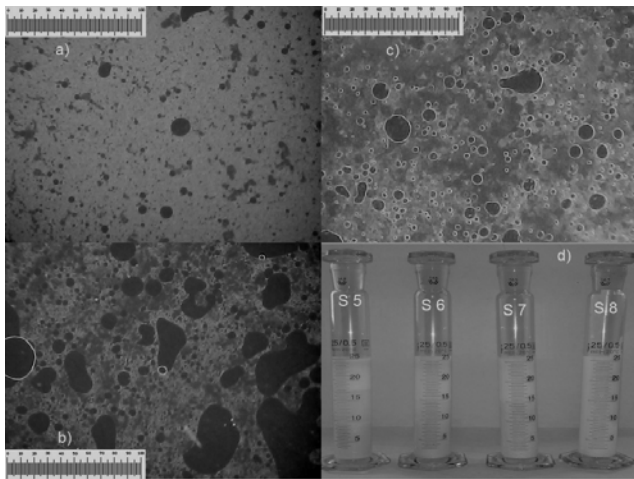


Fig. 2. Microscope pictures of sample 5. a) – emulsion w/o 24 h for preparation, b) – emulsion o/w 24 h for preparation, c) – emulsion 10 minutes after preparation, d) samples 5 – 8 with 5 % SI 24 h after preparation.

Graphical view of three phases of sample 5 presented in Fig. 3 into constructed phase diagram. Two types of emulsions were observed after first day of investigation. The water phase can be seen in the left region of the triangle phase diagram. On the other side, o/w emulsion occupies upper left region (water rich regions) of the phase diagram. The region w/o emulsion occupies the middle lower region (oil rich regions). The formation with or w/o emulsion depends on the composition of the protein, quantity of protein and its solubility in the oil and water. The results obtained in this work also show that areas of w/o emulsion as increasing the value of protein and oil. In some cases with appropriate composition, protein and oil has appeared in some gel structures. The investigation of the emulsion systems' purpose was to slow down phase separation in emulsions and decrease part w/o (water/oil) emulsion.

Thermodynamic parameters of emulsion samples Gibbs free energies, enthalpies and entropies have been calculated and their values are presented in Table 2. The classical thermodynamics equations have been used [20]. Negative Gibbs free energies indicates that the system is more stable. Due to this purpose, equilibrium constant has been estimated at all samples using spectrophotometer measurements of absorbance.

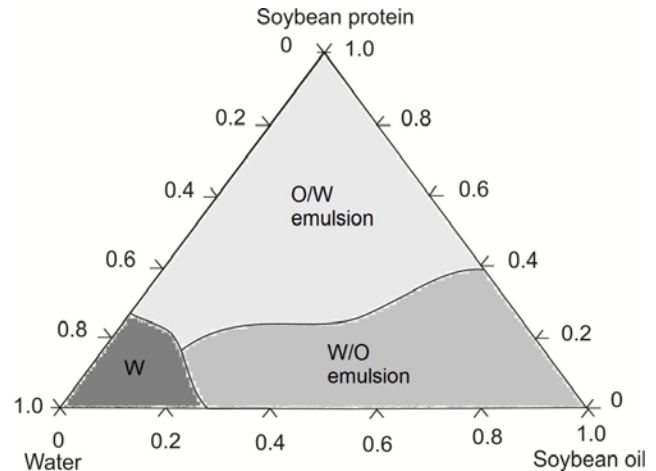


Fig. 3. Ternary phase diagrams of Soybean oil/Water/Soybean protein isolate of sample 5.

Water solutions with different emulsion concentration: 0.04, 0.08, 0.12, 0.16 and 0.2 mol dm⁻³ were prepared after preparation of emulsions. Absorbance of all emulsion solutions has been measured at 350 nm at room temperature. Equilibrium constants exhibit values from 1.919 to 6.108 (positive values). This conclusion is important, because in Van't Hoff equation includes lnK. The values less 1 (K<1) after logarithm had negative values, but in this case all K>1. When K<1 Gibbs free energy exhibits positive value and the process is impossible. The samples in this case have been destroyed in their preparation.

The thermodynamic stability determined by Gibbs free energy ΔG has been calculated by the following equation:

$$\Delta G = -RT \ln K \quad (1)$$

Where R is the universal gas constant (R = 8.314 J K⁻¹ mol⁻¹), T is the absolute temperature (K), ΔG is Gibbs free energy (kJ mol⁻¹), K – equilibrium constant.

The enthalpy of formation of emulsion is calculated by applying the Vant-Hoff equation.

$$\frac{d \ln K}{d(1/T)} = \frac{-\Delta H}{R} \quad (2)$$

ΔH is enthalpy of formation of emulsion (kJ mol⁻¹).

Once the Gibbs free energy and the enthalpy has been obtained, the entropy can be determined by using the classical thermodynamic equation (3):

$$\Delta S = \frac{(\Delta H - \Delta G)}{T} \quad (3)$$

ΔS are entropy (kJ K⁻¹ mol⁻¹).

Table 2. Thermodynamic parameters obtained in emulsion samples 1 – 16 at 298.2 K

Emulsion N	ΔG [kJ mol ⁻¹]	ΔH [kJ mol ⁻¹]	ΔS [kJ mol ⁻¹ K ⁻¹]
1	-3.688	-18.954	-0.052
2	-3.864	-19.022	-0.051
3	-4.178	-19.158	-0.050
4	-4.480	-19.289	-0.049
5	-3.068	-18.351	-0.052
6	-3.262	-18.759	-0.052
7	-3.536	-18.878	-0.051
8	-4.299	-19.209	-0.050
9	-2.556	-18.453	-0.053
10	-3.032	-18.659	-0.052
11	-3.953	-19.059	-0.051
12	-4.469	-19.284	-0.049
13	-1.598	-17.858	-0.056
14	-1.649	-18.037	-0.055
15	-1.186	-18.059	-0.055
16	-3.114	-18.695	-0.052

According to the obtained results of Gibbs free energy, all emulsions had negative values. This is a reason that it can be concluded that all processes in emulsions have been spontaneous ($\Delta G < 0$). The Gibbs energy is criterion for emulsion stability. When the ΔG increases in negative values the emulsions increase their stability. Enthalpy of formation exhibits negative values too and this is connected with exothermic process. Entropy in the emulsions exhibit very small values and these negative values connected with phase separation of emulsion system make it difficult to determined determine the direction of the process.

Fig. 4 presents Gibbs energy as dependent on mass of oil. The figure shows that the more mass of oil increases, the more Gibbs energy increases as negative values and systems is are more stable. Samples 1 – 4 with 4 % SI are more stable. Sample 4 exhibit the most Gibbs energy - 4.480 kJ mol⁻¹. The next sample which exhibits the similar result is sample 12 with 7 % SI. The Gibbs energy has been - 4.469 kJ mol⁻¹. The samples 13 – 16 with mixture of SI + casein have been less stable.

Their Gibbs energies being from - 1.598 to - 3.114 kJ mol⁻¹. These results have been confirmed in Fig. 5. This figure presented a microscope picture from sample 13, 10 minutes after preparation of emulsion, with the exception of lower Gibbs energy on this emulsion, the big droplets are seen after microscope observation. It is shown that this emulsion was the most unstable. The casein most likely has properties which decrease emulsion stability.

The electrolytes have characteristics that improve emulsion stabilities when added in small portions. The experiment has been performed in two samples 4 and 10.

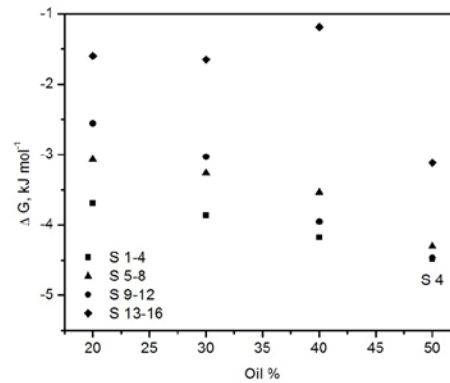


Fig. 4. Dependence between Gibbs free energy and mass % of Oil.

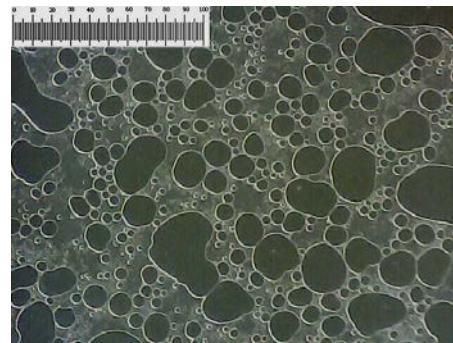


Fig. 5. Microscope pictures of sample 13, 10 minutes after preparation of emulsion.

Table 3. Investigation of emulsion stability in samples N° 4 and 10 with addition of NaCl: 1 – 5 %.

NaCl, %	ΔG [kJ mol ⁻¹]	ΔH [kJ mol ⁻¹]	ΔS [kJ mol ⁻¹ K ⁻¹]
Sample 4			
1	-4.073	-19.112	-0.050
2	-4.626	-19.352	-0.049
3	-3.737	-18.966	-0.051
4	-3.064	-18.674	-0.052
5	-2.990	-18.642	-0.053
Sample 10			
1	-3.296	-18.774	-0.052
2	-3.614	-18.913	-0.051
3	-3.435	-18.835	-0.052
4	-3.428	-18.832	-0.052
5	-2.488	-18.424	-0.053

Edition of NaCl has been increased from 1 to 5 %. Thermodynamic parameters of investigated samples have been calculated and are presented in Table 3. Sample 4 without NaCl has Gibbs free energy - 4.480 kJ mol⁻¹. Sample 4 with addition of NaCl has Gibbs free energy - 4.626 kJ mol⁻¹. With the increase of NaCl % the emulsion stability decreases. Sample 10 exhibits similar results.

With 2 % NaCl addition in emulsion sample, the emulsion is more stable.

4. Conclusions

In this study it has been found that emulsions with 4 % Soybean protein isolate and a high amount of soybean oil have exhibited good emulsion stability. These results are confirmed through calculated thermodynamic parameter ΔG , ΔH and ΔS . A ternary phase diagram of one sample has been constructed. Further investigations will be required to improve emulsion stability of emulsion systems. This phase diagram study provides valuable information on the role that the structure of emulsions, water and oil phases plays in determining the properties of the system at presented any composition. These investigations have been used for preparation in some specific emulsions. These emulsions can be applied in food industries and in some meat products.

Acknowledgments

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