



## Research Article

# Kinetic investigation of emulsion stability in oil/water emulsions stabilized with protein

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### Abstract

The emulsion stability of soybean oil/water (O/W) with different concentrations was investigated. The dynamics of emulsion stabilities were investigated from 1 to 15 days as a measure of turbidity. Three series of samples were prepared with 2 %, 4 % and 5 % soybean protein isolates (SI). Obtained results were compared with obtained optical microscope. It was found that emulsions exhibited more stability in samples with 2 % SI. The pH was measured in all emulsions. pH interval was between 5.8 and 7.4 and after analysis the emulsions exhibited high stability with pH values of 7.2-7.3.

**Keywords:** emulsions, soybean oil, soybean protein isolate, spectrophotometry

### Introduction

Flavor and color of food products are often generated by emulsification process between components in a water phase and oil phase. Emulsions are thermodynamically unstable systems that tend to breakdown during storage. Emulsion stability connected with breakdown flocculation, coalescence, sedimentation, creaming, Ostwald ripening or phase inversion (McClements, 2005). A wide variety of oils are used in the preparation of different emulsions. The oils are used in different soups, salad dressings and others. Soybean oil and soybean protein isolate (SI) are appropriate compound to prepare emulsions for food industries (Krog *et al.*, 1983; Jaynes *et al.*, 1983; Dickinson *et al.*, 1982; 1995; Swaisgood, 1996). The soybean oil-in-water emulsion using nonionic Tween series surfactants were investigated (Hsu *et al.*, 2003). The influence of pH and electrolyte on the  $\zeta$  potential of emulsion drops was examined and found that  $\zeta$  potential exhibited strong dependence on pH. The emulsion turbidity as function of particle size and concentration were used to determined emulsion stability by measuring the change in turbidity with time (Reedy *et al.*, 1981). The possibility of producing stable oil-in-water (O/W) emulsions containing oil droplets surrounded by multiple layer of interfacial membranes from food grade ingredients were investigated. The droplets in these emulsions had good stability for aggregation over a wide range of pH values and salt concentrations (pH 4–8 at 0 mM NaCl and pH 3–8 at 100 mM NaCl) (Satoshi *et al.*, 2004). Formulation of water-in-virgin coconut oil (w/o) microemulsions and determine its stability were investigated (Rukmini *et al.*, (2012). These microemulsions were subjected to stability tests which include centrifugation, heating treatment and storage at room temperature. The emulsions remained stable during storage, even after centrifugation, but they were not stable when subjected to heating at 70 °C or higher. The stabilizing effect of spruce galactoglucomannan (GGM) on a model beverage emulsion system was studied (Mikkonen *et al.*, 2009). The initial turbidity increased with increasing GGM content, but after 14 days storage at room temperature, the turbidity was the highest for GGM/oil ratio of 0.10:1 when ethanol-

precipitated GGM was used. Increasing the storage temperature to 45 °C led to rapid emulsion breakdown, but a decrease in storage temperature increased emulsion stability after 14 days. A low degree of polymerization and a high degree of substitution of the modified galactomannans were associated with a decrease in emulsion turbidity. The objective of this work was to investigate experimentally the emulsion stability of oil/water emulsions stabilized with protein continue in time at storage between one and fifteen days.

### Materials and methods

#### Chemicals

The soybean oil was used for preparation of emulsions. The emulsions O/W were prepared as follows: the SI 2, 4 and 5 % were soluble in water and added soybean oil (% water indicates in Table 1). The emulsions were prepared with homogenizer.

#### Determination of pH

pH in samples was determined by pH meter Hanna HI 98127 with Replaceable Electrode. The pH values of the final 15 emulsion samples were between 5.8 and 7.4. Citric acid was used for correction of pH value of emulsions.

#### Microscopic study

All emulsions were investigated with digital microscope Brasser. Digital microscopes, like the Bresser Junior USB Hand-held Microscope, are powered the USB port on PC. The images from the MikroCam MP Microscope camera were prepared.

#### Determination of emulsion turbidity

The investigations were performed with Double Beam UV/VIS spectrophotometer Camspec M550. The measurements were made in down phase, because after first day, the emulsions separate on two phases: up - oil phase and down water phases. The turbidity of emulsions measured from water phases (Hsu *et al.*, 2003), for each

sample turbidity was determined at 380 nm wavelength.

Statistical analysis

The results reported in the present study are the mean values of at least three determinations and the coefficients of variation were found to be below 2% in all cases. Linear regression analysis was performed using the statistical package of Orijin 7.0.

Results and discussion

The 15 emulsions were prepared with different mass % oil/water and 2, 4 and 5 % SI. The chemical composition of emulsions and pH values are presented in Table 1.

Table 1. Initial composition of samples

N	Soybean oil (%)	Water (%)	SI (%)	pH
1	10	88	2	7.22
2	10	86	4	7.35
3	10	85	5	7.28
4	15	83	2	7.36
5	15	81	4	5.91
6	15	80	5	6.02
7	20	78	2	5.99
8	20	76	4	5.85
9	20	78	5	5.76
10	25	73	2	5.83
11	25	71	4	5.86
12	25	70	5	6.08
13	30	68	2	6.54
14	30	66	4	6.32
15	30	65	5	6.87

Table 2. Measured turbidity of emulsion 1 between 1 and 15 days

Time, days	$\tau \pm 0.003, \%$
1	5.545
3	5.218
6	4.916
8	5.098
10	5.372
13	5.524
15	4.393

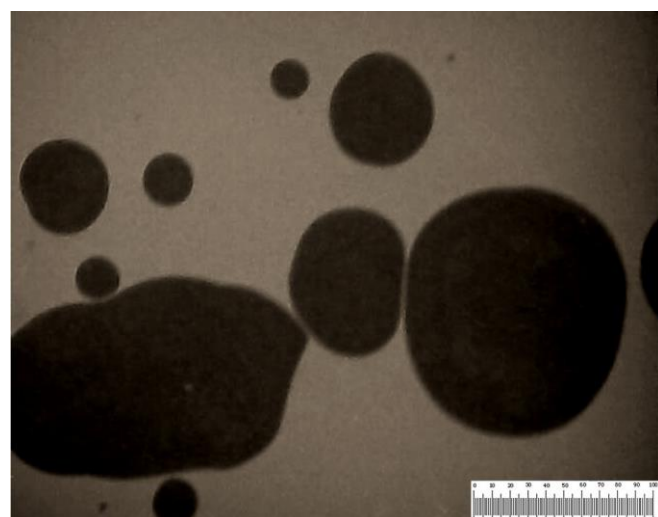


Figure 1. Picture of sample 1 observed 8 days after preparation of emulsion by optical microscope

All emulsions were observed by optical microscope after their preparation and pictures of all samples were

taken. After that the observations were done in fourth, eighth and twelve days from continues phase. The size of the detected particles did not significantly change during the observation time. The processes coalesce and others finished in emulsions after 24 h. Observations with optical microscope showed that the average particle size was approximately above 1 mm. The results indicated that if oil droplets combined due to emulsion breakdown by coalescence or flocculation.

Figure 1 presented micrograph picture of sample 1 prepared with optical microscope after 8 days of storage. The figure shows large droplets of emulsion connected with processes during the experimental days. Large particle size presented unstable emulsions. In Table 2 represents experimental results of turbidity of emulsion 1 by spectrophotometer measurements. The measurements showed that observed middle turbidity in all days and this results presented stable emulsion. Measurement of turbidity of emulsions were used to calculate particle size and compared with these results of optical microscope.

Figure 2 presented initial turbidity of emulsion samples 1 - 6 measured at different days. First turbidity measured after the preparing of emulsion was high and after 24 hours the turbidity decreased. In presented samples observed two visibility phases after first day: one upper cream phase and one down water (turbid) phase. Two phases were observed and determined as two types of emulsions. Water phase determined as emulsion oil in water (O/W), but cream phase as emulsion water in oil (W/O). Sample 1 (Figure 3) prepared with 2 % SI exhibited middle turbidity in first day and no more change of values in other days. Samples 4 and 5 presented in the same figure showed the turbidity decreased around 6 days. According to the values of turbidity the emulsion sample 1 was stable for long time.

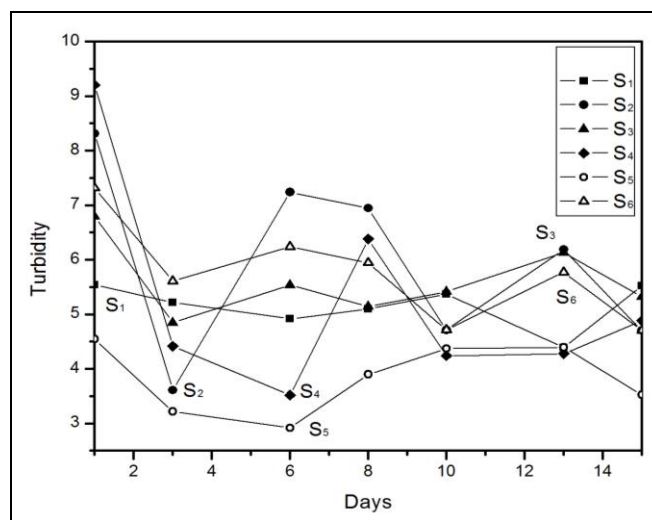
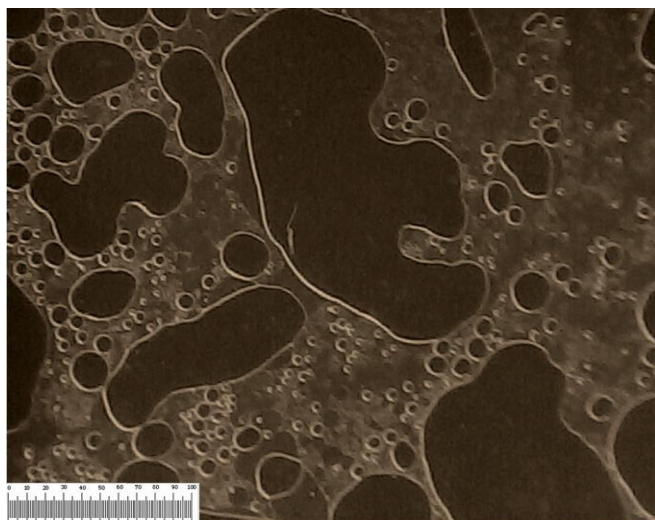


Figure 2. Effect of time (in days) on the turbidity of emulsions stabilized by different SI in emulsion samples 1 – 6

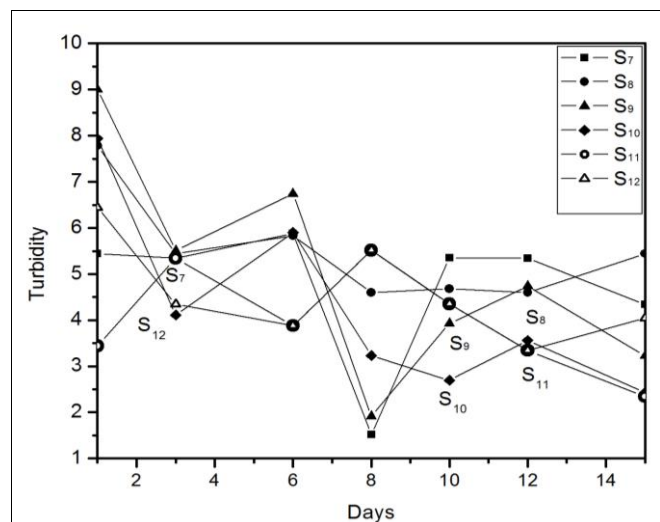
Figure 3 presented micrograph picture with optical microscope preparation of emulsion in sample 9 after 8 days. In the figure can be seen that droplet of emulsion have different sizes and different shapes. Emulsions prepared with 5 % SI are unstable compared with others. Table 3 presented experimental results after investigation of emulsion 9 by spectrophotometer measurements. In this case emulsions exhibited small size to first 6 days. After that the particle size increased.

**Table 3.** Measured turbidity of emulsion 9 between 1 and 15 days

Time, days	$\tau \pm 0.005, \%$
1	9.003
3	5.512
6	6.744
8	1.916
10	3.926
13	4.744
15	3.226



**Figure 3.** Picture of sample 9 observed 8 days after preparation of emulsion by optical microscope

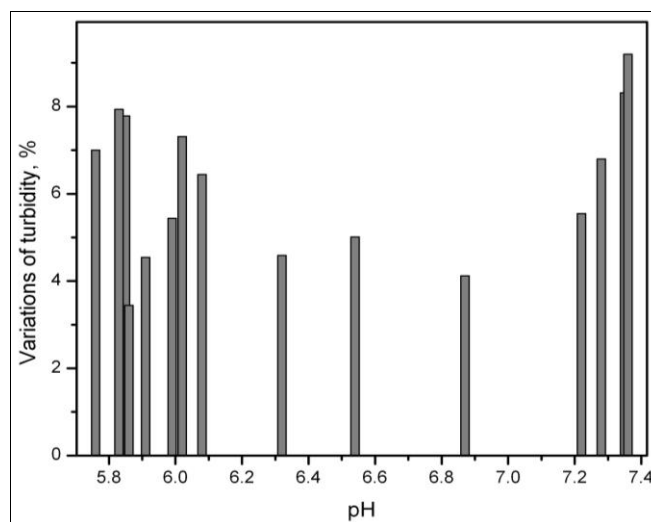


**Figure 4.** Effect of time (in days) on the turbidity of emulsions stabilized by different SI in emulsion samples 7 – 12

The similar results were observed for emulsion samples 7 – 12 in Figure 4. Immediately after preparation of each emulsion the turbidity was measured. The turbidity of emulsions 7 and 8 in first day presented stable emulsions. After that turbidity were measured from 1 - 15 days. A larger was turbidity connected with smaller particle size. In the last days the turbidity decrease and the particle size increased. Around 8 days all samples exhibited decrease in turbidity and after that emulsions exhibited one constant values of turbidity. When the emulsions were unstable, the turbidity decreased immediately. These decreases may be due to the coalescence of droplets and correlated with the observation of phase separation. According to the

experimental results, microscopic observations connected with this prepared with turbidity measuring and samples with smaller quantity of SI were more stable.

Figure 5 presented dependence between variation of turbidity and pH values. The emulsions with high pH (S1-S4, Table 1) exhibited larger turbidity. This is proof for increased stability of emulsions.



**Figure 5.** Variation of absorbance at S1 - S15 as dependence of pH

**Conclusion**

The interactions between soybean protein isolate and other components were studied to understand the stability of food emulsions. The emulsion stability was determined by optical microscope, measured of turbidity of emulsions and by determined particle size. Finally the all results – particle size, change of turbidity and different pH show that the emulsions prepared with 2 mass % soybean protein isolate and pH = 7.2-7.3 exhibited increase of emulsion stability.

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