Effect of Salt on the Stability of Vegetable Oil-in-Water Emulsions Stabilized by Soybean Protein and Microgel

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Summary: The preparation of vegetable oil-water emulsions stabilized by soybean protein and microgel is described. The soybean protein was obtained from *n*-hexane-defatted soybean powder using a Soxhlet extractor. Using equal volumes of oil and water, vegetable oil-water emulsions were formed either by handshaking the mixture or homogenizing the mixture using a Lab homogenizer. The emulsion was characterized using a drop test and microscopy observation. The drop test shows that the preferred emulsion is vegetable oil-in-water (o/w). The effect of salt and emulsifier concentration on the stability and emulsion drop size was investigated. Emulsions stabilized by soybean protein without addition of salt breakdown after 3 days of preparation because of decomposition of the protein. For emulsions stabilized by microgel in the absence of salt, phase separation occurred within 1 hour. At a fixed salt concentration, it was found that increasing the emulsifier concentration has a significant effect on the stability and drop size of the emulsions stabilized by both protein and microgel. For emulsions stabilized by soybean protein, the stability of emulsions increased with increasing salt concentration without any significant influence on the drop size. The results obtained from the surface tension measurement revealed that different mechanisms of stabilization exist in emulsions stabilized by the protein and microgel.

Keywords: Emulsion, Soybean protein, Microgel, Concentration, Stability.

Introduction

Emulsions are a class of dispersed systems consisting of a mixture of two immiscible liquids where one of the liquids is dispersed as small droplets in the other liquid [1, 2]. Many industrial products are dispersed systems made from two immiscible liquids and their stability depends on the emulsifier used in the formulation. For example, milk is an oil-in-water emulsion stabilized by casein and margarine is a water-in-oil emulsion stabilized by fatty acid crystals [3, 4]. After emulsification is completed, the properties of an emulsion tend to change with time through some breakdown processes which eventually affect their stability. To prevent emulsion droplets from breaking, a surface-active material or an emulsifier is usually needed. Emulsifiers are substances that are used for stabilizing emulsions by increasing their kinetic stability. Based on their mode of action, emulsifiers can be classified into three, namely surfactant, polymer and particles. Surfactants are amphiphilic molecules that are surface-active. When a surfactant is added to two immiscible liquids, it adsorbs at the oil-water interface, lowers the interfacial tension and imparts needed stability to the system. Polymers are macromolecules consisting of many repeating units called monomers. Polymer molecule possessing sufficient surface activity can act as an emulsifier. In this case, the polymer molecules adsorb at the interface to form structured interfacial films that prevent the breaking down of emulsion drops. The third type of emulsion stabilizer is solid particles. The mechanism of stabilization is based on the strategic adsorption of the solid particles at the interface. The surface activity of these particles is not necessarily due to their amphiphilic nature [5, 6]. The solid particles coat emulsion droplet surfaces and form rigid support that prevents the breaking of emulsion droplets. Such an property can be attributed to the partial wettability of the particles at the oil–water interface. For a particle to be an effective emulsifier, it must be partially wetted by both liquid phases as described by Finkle *et al* [7]. The wettability of particles can be quantified by the oil–water contact angle. The stabilization of emulsions has been achieved using some model organic or inorganic particles, such as silica [8], pigment particles [9], cellulose microparticles or nanocrystals [10, 11], chitin nanocrystals [12], modified starch particles [13-15], flavonoid particles [16], solid lipid nanoparticles [17] and zein protein particles [18].

One of the ongoing research interests is the development of cheap food-grade emulsifiers that can be used to formulate consumable products. For almost a decade, the nutritional, as well as economic importance of soybean proteins, has been known as a classical example of plant proteins suitable for human consumption because of their good nutritional value,

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digestibility, processability and biological functionality [19]. Unlike cereal crops which have inadequate protein content and unbalanced amino acid composition, soybean products are rich in essential amino acids that are necessary for human growth and healthy living. The consumption of soybean protein can cause a significant reduction in total cholesterol in humans. Soybean protein is made up of ∝-conglycinin and glycinin. ∝-conglycinnin is an example of a glycoprotein. It is a trimer that consists of three major subunits. One of its subunits has a cysteine residue near the N-terminal. Glycinin consists of two polypeptide parts that are linked together by a disulfide bond [20].

Reports describing the stabilization of emulsions using soybean protein are available in the literature [20, 21]. In some of these reports, the soybean protein is used in conjunction with other proteins [22], fibre [23], carbohydrates [24], *etc*. The emulsifying ability of soybean protein isolate particles has been investigated by Liu *et al* [25]. The protein particles are generated by combined treatment of heating and electrostatic screening. In a report from Huang *et al*. [23], the effect of adding insoluble soybean fibre to emulsion stabilized by low concentration of soybean protein isolate was studied. They ascribed the enhanced stability of the emulsions to electrostatic interactions and the gel-like network structure which facilitated the adsorption of protein at the interface, providing mechanical support against coalescence. In this research work, the effect of salt on o/w emulsions stabilized by soybean protein and microgel is studied. Emulsions were characterized by drop test, macroscopic visualization and optical microscopy observation. The influence of emulsifier concentration on emulsion stability and drop is investigated. From the results obtained from surface tension measurement, we propose mechanism for the stabilization of emulsions for system containing soybean protein and microgel.

Experimental

Materials

Fresh Soybean samples were obtained from Oye market, Oye-Ekiti, Ekiti State, Nigeria. The powder was obtained by grinding the soybean with an electric blender. Thereafter, the sample was stored in a plastic container for further use. The chemical reagents used are hydrochloric acid, Sodium hydroxide, Sodium chloride, *n*-hexane, ethanol, distilled water and vegetable oil. The vegetable oil was purchase from the market. All the chemical reagents used are of analytical grade.

Methods

Procedure for defatting the soybean powder

40 kg of soybean was pulverized with an electric blender. 30 g of the pulverized sample was wrapped in a white clean cloth and placed inside the Soxhlet apparatus. The round bottom flask was filled up to two-thirds of its capacity with *n*-hexane. The Soxhlet extractor was then fitted with the refluxing condenser and the system was heated gently and was left to siphon for 8 hours. The defatted powder was removed from the Soxhlet apparatus and was kept in an oven to dry at a regulated temperature of 40 °C for 3 days.

Determination of Proximate composition of soybean flour

The proximate parameters that were determined include moisture content, crude fibre, crude fat, protein and carbohydrates. The proximate analysis was carried out using the following procedures outline below [26, 27].

(i) Determination of ash content

The total ash content is the amount of non-volatile residual substance obtained after the complete calcination of a sample. 3g of the soybean powder was put into different 3 marked crucibles of known weight. The samples were placed in an oven at 600 °C for 24 hours. The crucibles plus the content were reweighed after cooling. The percentage of ash content (AS) was calculated by

$$\%AS = \frac{MC}{PE} \times 100 \qquad (1)$$

where PE is the mass of the test sample and MC is the mass of the sample after ashing.

(ii) Determination of moisture content

The moisture content was determined by the drying method. It is a method that is mostly used for estimating the moisture content of the sample. It is based on the weight loss of water after heating under standardized conditions in an ovum at 105 °C. A cleaned and dried petri dish was weighed, and its weight was recorded as (W_1). 5 g of the sample was weighed into the dish. The petri dish and the sample were weighed and recorded as (W_2) The Petri dish with the sample was then transferred into the thermosetting oven maintained at 105 °C for about 3 hours. It was later transferred into the desiccator for

effective cooling and then reweighed. This process was performed repeatedly until a constant weight (W_3) was obtained. The loss in weight during drying in percentage was taken to be the percentage of moisture content.

$$\%\ moisture\ \ \frac{W_2-W_3}{W_2-W_1} \times 100 \qquad (2)$$

(iii) Determination of Crude Protein (Using Kjeldahl Method)

The Kjeldahl method was used to determine the amount of crude protein in the soybean sample. 1 g of the sample was weighed into a Kjedhal flask and 10 ml of H_2SO_4 with Kjedhal catalyst was added. The mixture was then heated on a heating mantle while the flask was rotated at intervals until the digestion was completed. This was allowed to cool and the digested sample was made up to 50 ml solution (V_1). Thereafter, 25 ml of the resulting solution was pipetted into a clean flask and neutralized with 50 ml of 40 % sodium hydroxide. Then titration was performed using 2% boric acid to determine the amount of ammonia. The percentage of nitrogen was determined by:

$$\%\ Nitrogen = \frac{(T-B)\times 14\times 0.01\times V_1}{weight\ of\ sample\ \times V_2} \times 100 \quad (3)$$

where T is the titre value, B is the blank, V_1 is the total volume of digested sample and V_2 is the volume of digested sample used for titration. The amount of crude protein contained in soybean is obtained by multiplying the nitrogen content of the sample by 6.25.

(iv) Determination of crude fat

The crude fat was determined using a Soxhlet extractor. A previously dried white clean cloth was weighed as (W_1). 2.5 g of the sample was weighed into the white cloth (W_2). This was tightened very well with white thread and transfer into the Soxhlet extractor. A 500 ml round bottom flask was filled up to two-thirds of its capacity with *n*-hexane. The Soxhlet extractor was then fitted with a refluxing condenser and the heat source was adjusted so that the solvent boils gently. The system was left to siphon for 8 hours, after which the solvent with the oil was removed. The white cloth and defatted samples were dried in the oven at 40 °C for about 3 days. The sample was allowed to cool down in the desiccators and weighed as (W_3). The percentage of fat content was calculated thus;

$$\%\ crude\ fat = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad (4)$$

(v) Determination of crude fibre

Crude fibre is the remaining organic component when the defatted sample has been successfully treated with diluted acid (H_2SO_4) and dilute base (NaOH). Crude fibre is the indigestible portion of any substance. It is known that fibre consists of cellulose, which can be digested to considerable extents by both ruminants and non-ruminant animals. The determination of fibre content in plant tissue provides a distinction between the most digestible crude fibre. About 3 g (W_1) of the defatted sample was weighed into a 500 cm^3 conical flask and then 200 cm^3 of 1.25% of H_2SO_4 was added to the sample in the conical flask already placed on the heating mantle. The system was heated strongly to boil for 2 min and thereafter allowed to boil gently for about 30 min. The mixture was filtered through Whatmann filter paper and rinsed well with hot distilled water. The sample was scrapped back into a flask containing 200 cm^3 of 1.25% NaOH with a spatula, and it was heated gently for 30 min. Thereafter, it was filtered through Whatmann filter paper and rinsed well with hot distilled water four times and once with 10% HCl to neutralize the NaOH remaining in the sample. Thereafter it was rinsed with hot distilled water for four times and twice with ethanol. The residue was scrapped into a crucible and dried in a thermosetting drying oven at 105 °C. Then, the sample was reweighed (W_2). The percentage of crude fibre was calculated using

$$\%\ crude\ fibre = \frac{W_2}{W_1} \times 100 \qquad (5)$$

(vi) Determination of carbohydrate content

Carbohydrate is the most abundant constituent of plants and animals. The most approach for the determination of carbohydrate content of food is the difference between the total predominant content in percentages (ash, crude protein, fat, crude fibre, moisture) and one hundred.

% Carbohydrate = 100 – (% ash + % crude protein + % fat + % crude fiber + % moisture)

Procedure for the extraction of soybean protein

1 g of the defatted soybean flour was weighed into a 100 cm^3 beaker and 10 cm^3 of water was added to it. The powder was dispersed in the water by stirring the mixture and thereafter the pH was adjusted to 8 by adding drops of 2 M NaOH solution. The suspended particles were removed with a sieve and the fine particles were separated by centrifuging at 10,400 g for 15 min at 20 ºC. The supernatant was carefully transferred into a beaker and the solution pH was

adjusted with 1 M HCl solution until a white precipitate was formed at around pH 4.5 [28].

Preparation of soybean protein microgel

The soybean protein microgel was prepared using the previous reported top-down technique with a little modification [29]. After the extraction of the protein, the dispersion was heated in a water bath set at a temperature of 90 ºC for about 1 hour. The system was allowed to cool down and thereafter the resulting solution was stirred vigorously using a Hanchen Lab homogenizer with a 12 mm head, operating at 16,000 rpm for 2 min. This resulting dispersion was stored in a capped vial and was used for making emulsions.

Characterization of soybean protein and microgel

Biuret Test

To 2 cm^3 of the protein sample, 3 cm^3 of 10% NaOH solution was added. Then followed by 4-5 drops of 0.5% copper sulphate solution. The resulting solution was mixed thoroughly. The appearance of the violet colour confirmed the presence of protein.

Fourier transformation infrared (FTIR) analysis

The FTIR analysis was performed using an FTIR spectrometer (SHIMADZU FTIR-8400S). Both soybean protein and microgel were analyzed. The samples were mixed with analytical grade KBr at a weight ratio of 5/200 mg. The reason for this measurement is to establish the different functional groups in the samples.

Optical microscopy observation

The aggregated structures formed by the soybean protein and microgel dispersions were probed by Bresser optical microscope at ×5 and ×10 magnifications and the digital micrographs of 1280 × 720 pixels were taken using a CCD digital camera system. The images were processed with a top view image software.

Quantification of the microgel

The mass of 1 cm^3 of the protein dispersion was determined gravimetrically. The mass of the dry empty filter paper was determined. Thereafter, 1 cm^3 of the freshly prepared soybean protein was measured into the filter paper placed in a funnel. Then the residue was dried in the oven at 50 ºC for 1 day and thereafter it was kept in the desiccator for 6 h. Then the mass of the filter paper plus sample was taken. The above procedure was repeated three times and an average mass was obtained.

Measurement of surface and interfacial tensions.

The surface tensions of pure water, oil and aqueous dispersions containing 0.105 g of soybean protein or microgel were measured using a ring method (BZY 102 Automatic Surface tensiometer). Before measurement was done, the tensiometer was calibrated using the standard mass. Thereafter the platinum ring was hung on a hook in the instrument. The measuring vessel was placed in a plastic jacket on a perpendicularly moveable platform. For the measurement of air-water (or oil-air) surface tension, the ring was immersed in the vessel containing distilled water (or oil). Then the ring was pulled out by lowering the platform slowly creating a meniscus at the air-water (or oil-air) interface and the surface tension was then recorded using the maximum-pull method. The surface tension of water was calculated using the software supplied by the manufacturer. Each measurement was reproduced at least 5 times to obtain an average. For the oil-water interfacial tension, 2 cm^3 of water was measured and then 2 cm^3 of the oil was added. The mixture was allowed to completely phase-separated and then the above procedure was followed to determine the interfacial tension [30].

Preparation of emulsion stabilized by soybean protein and microgel

Emulsions were prepared using equal volumes of oil and water. For emulsions stabilized by soybean protein, 5 cm^3 of soybean protein dispersion was measured into a clean screw-capped vessel and 5 cm^3 of pure vegetable oil was measured into the same screw cap vessels and the resulting mixture was hand-shaken vigorously for 2 min. For emulsions stabilized by soybean protein microgel, the above procedure was followed except that the mixture was homogenized using a Hanchen Lab homogenizer with 12 mm head, operating at 12,000 rpm for 2 min.

Characterization of emulsions

The drop test was carried out to confirm emulsion type by observing what happened when a drop of the emulsion was placed on the surface of 2 cm^3 of either pure oil or water. Water continuous emulsions dispersed in water and remained as drops in oil (*i.e*. o/w emulsion) and *vice versa* [31]. The stability of o/w emulsions to creaming was assessed by monitoring the increase with time of the position of the clear water (serum)-emulsion interface, whereas the extent of coalescence was estimated from the movement of the oil-emulsion boundary. The fraction of oil or water released is calculated by dividing the amount of oil or water released by the total volume of oil or water used for the preparation of emulsions. Optical microscopy observation of emulsion was performed with a Bresser optical microscope at ×4 and ×10 magnifications and the

digital micrographs of 1280 × 720 pixels were taken using a CCD camera system. The images were processed with a top view image software. The microscope was calibrated using a reference stage graticule. A drop of the emulsion was taken from the centre of the emulsion layer and diluted with the appropriate continuous phase. The average diameter of the droplets was determined with Image J 1.47v by measuring the size of 100 droplets in the digital micrographs.

Results and discussion

Proximate Composition

The results of the proximate composition of soybean powder are given in Table 1. The results indicate that a high percentage of carbohydrate (55.36%), and a significant amount of moisture (15.36%) and protein (21.23%) is present in the soybean powder. The percentage of protein obtained here is similar to that reported for some soybean genotypes in ref. 27. The values obtained for carbohydrates and crude fibres are close to those reported for genotypes, NGB 00113 and TGX 923-2E respectively in ref 27. The results also show a moderate amount of crude fat, fibre and ash content. The high moisture content could be attributed to the lowland rainforest areas where the soybean plants are grown.

Table 1: Percentage proximate composition of soybean powder.

Parameters	Proximate composition (%)
Ash content	2.40 ± 0.07
Moisture content	15.36 ± 0.31
Crude protein	21.23 ± 0.31
Crude fat	2.54 ± 0.03
Crude fiber	3.34 ± 0.03
Carbohydrates	55.13 ± 0.01

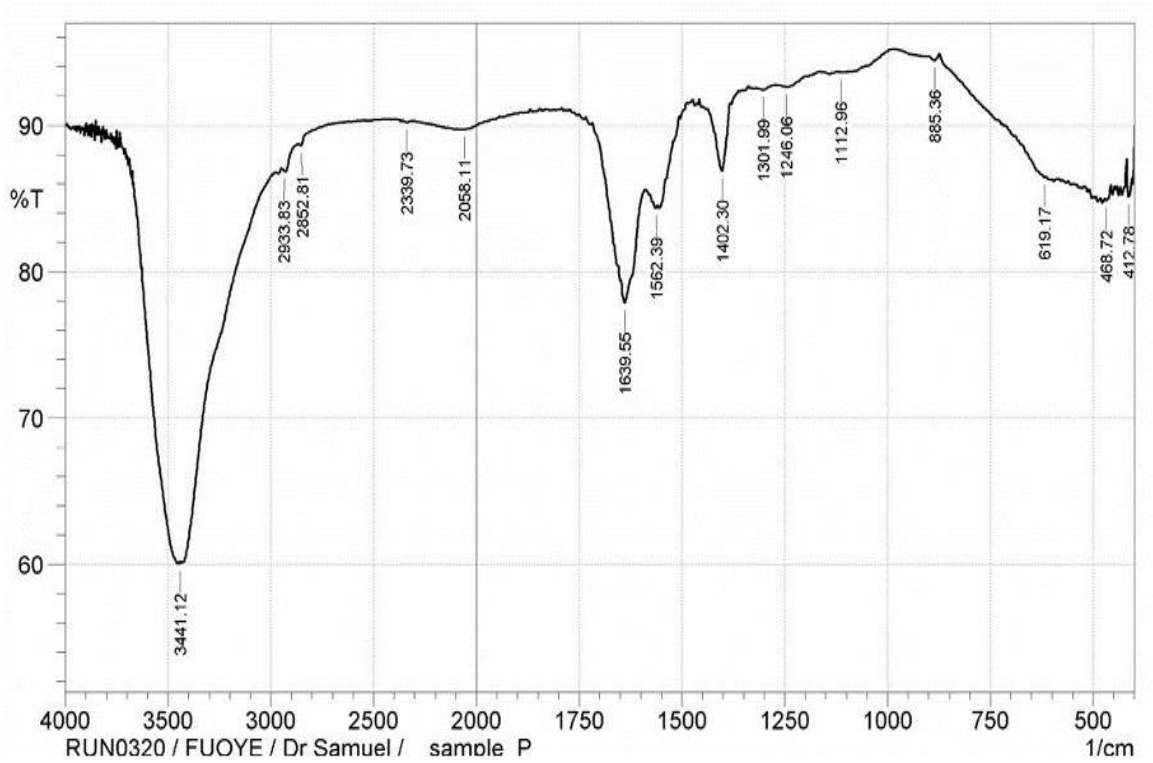
Characterization of soybean protein and microgel aqueous dispersions

The positive result obtained from the Biuret test confirmed the presence of protein in the soybean extract**.** The functional groups present in the soybean protein isolate was confirmed by FTIR analysis. The FTIR spectra of soybean protein and microgel are given in Fig 1. In Fig 1(a), the broadband near 3,441 cm^{-1} is for N–H stretching vibration and the peak at 2,933 cm^{-1} is assigned to O–H stretching vibration. The absorption at 1,633 cm^{-1} is assigned to C=O stretching. A peak at 1,301 cm^{-1} corresponds to O–H asymmetric deformation. The sharp absorption peak at around 1,112 cm^{-1} is due to C–O stretching vibration. The shape peak at 1,402 cm^{-1} is due to the C–N stretching vibration. The band at 2,852 cm^{-1} is due to the S–H stretching vibration of the cysteine amino acid [21] repeating unit in the protein molecule and the absorbance band at 885 cm^{-1} may be due to S–H asymmetric deformation vibration. The peaks around 468 cm^{-1} and 619 cm^{-1} may due to –S–S– and C–S respectively. The shape peak at 1,562 cm^{-1} is due to N–H asymmetric deformation. This spectrum confirms the presence of protein in the soybean extract. The FTIR spectrum in Fig 1(b) is for the soybean protein microgel. As seen in this spectrum, most of the peaks of absorption occur at a relatively lower frequency compared to that of the native protein. The absence of peaks at 2,852 cm^{-1} and 885 cm^{-1} in this spectrum may be due to the formation of the disulphide (–S–S–) bond as the protein undergoes gelation [30].

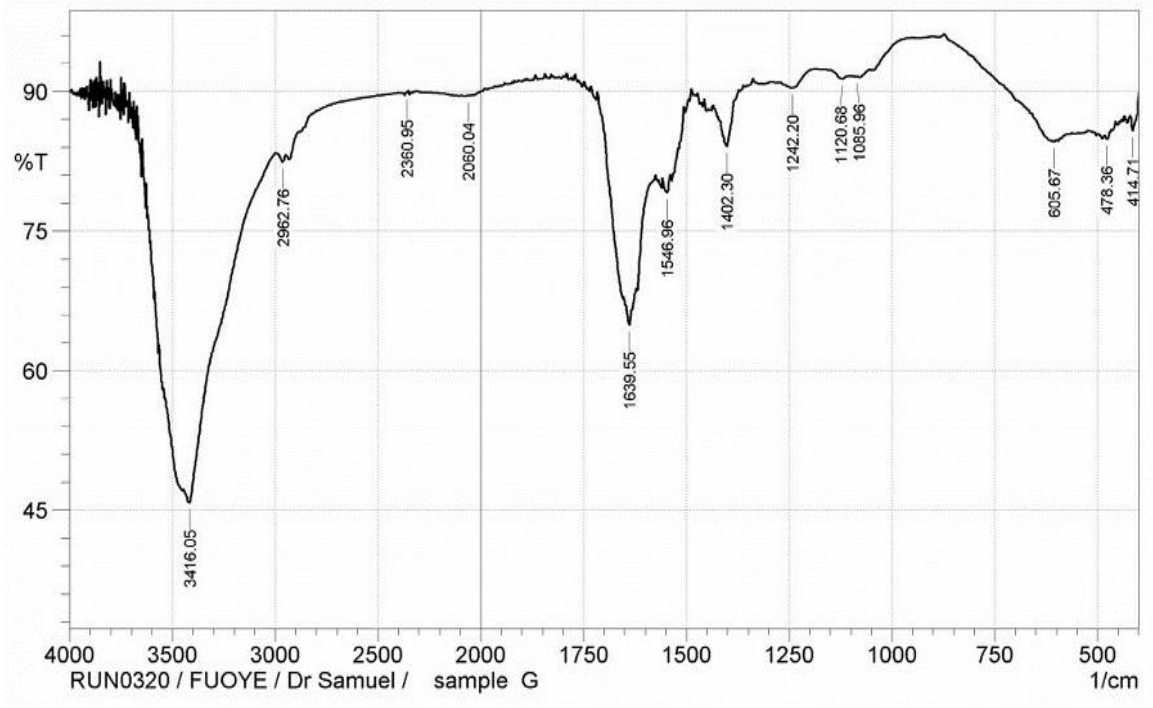
The aggregated structures formed in aqueous dispersions by soybean protein, hydrogel and microgel were probed by using an optical microscope. The micrographs thus obtained are given in Fig 2. As seen in Fig 2(a)**,** before gelation, soybean protein in the dispersion is slightly aggregated. The image shown in Fig 2(b) confirmed the formation of hydrogel upon the application of heat to the protein sample. The image reveals the formation of a highly flocculated and compacted structure. The optical microscopic images in Fig (c, d) were those obtained at different magnifications after the hydrogel was homogenized at 16,000 rpm for 2 min. A drastic change in the morphological structure of the dispersion is evident in the digital images. Polydisperse quasi-spherical microgels or soft particles are seen in the micrograph with an average diameter of 2.0 ± 0.8 μm.

Emulsions stabilized by soybean protein and microgel without salt

Emulsions of equal volumes of oil and water (ϕ_w = 0.5) at pH 4.5 were prepared without the addition of salt using either 0.52 g of soybean protein or microgel as an emulsifier. Emulsions stabilized by soybean protein and microgel are all oil-in-water (o/w). Emulsions were made by handshaking the mixture of oil and water for 2 min. For emulsion stabilized by microgel, the system phase-separated after 1 hour of preparation while emulsion stabilized by soybean protein remained stable for some days. Photo of the vessel containing emulsions 24 hours after preparation of a system stabilized by soybean protein is given in Fig 3 (a). As can be seen, the systems creams ejecting an aqueous layer below and no oil is seen above emulsions because it is stable to coalescence. After 4 days, this emulsion begins to breakdown because of the decomposition of the protein with the evolution of irritating smell. The microscopic image of the emulsion taken immediately after preparation is shown in Fig 3(b). The oil droplets in the micrograph are spherical, slightly flocculated and polydisperse with a mean drop diameter of 46 ± 20 μm.



(a)



(b)

Fig. 1: FTIR spectra of (a) soybean protein and (b) microgel.

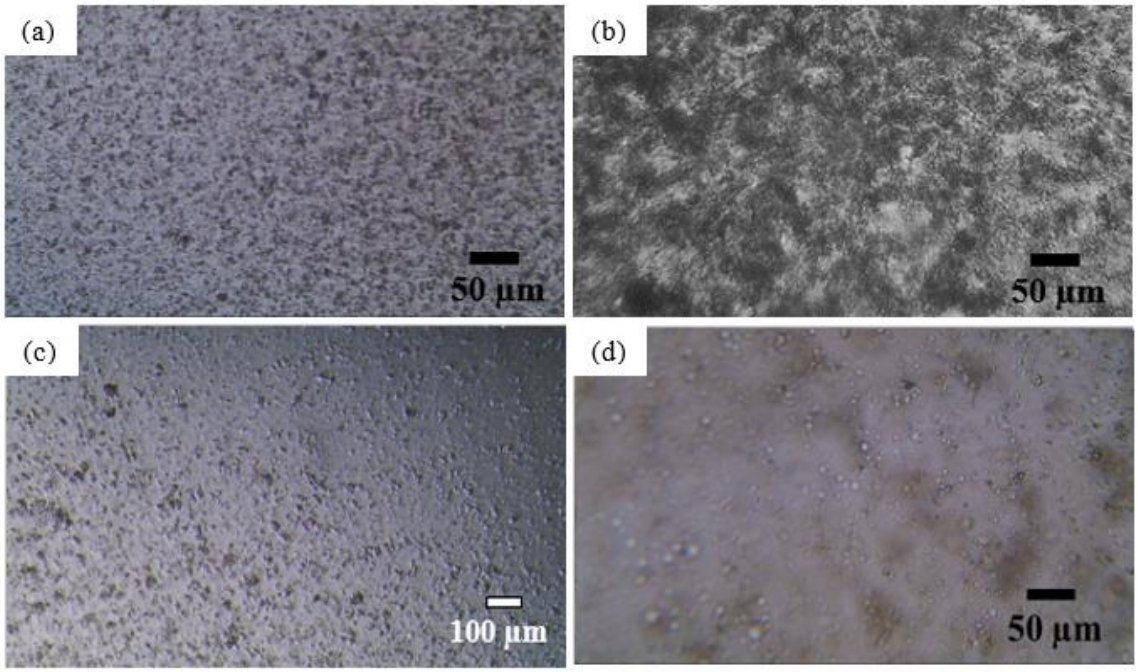


Fig 2: Optical micrographs of (a) 0.105 g of soybean protein dispersion, (b) 0.0105 g soybean protein hydrogel, (c,d) a dispersion containing 0.105 g of soybean protein microgel taken at different magnifications.

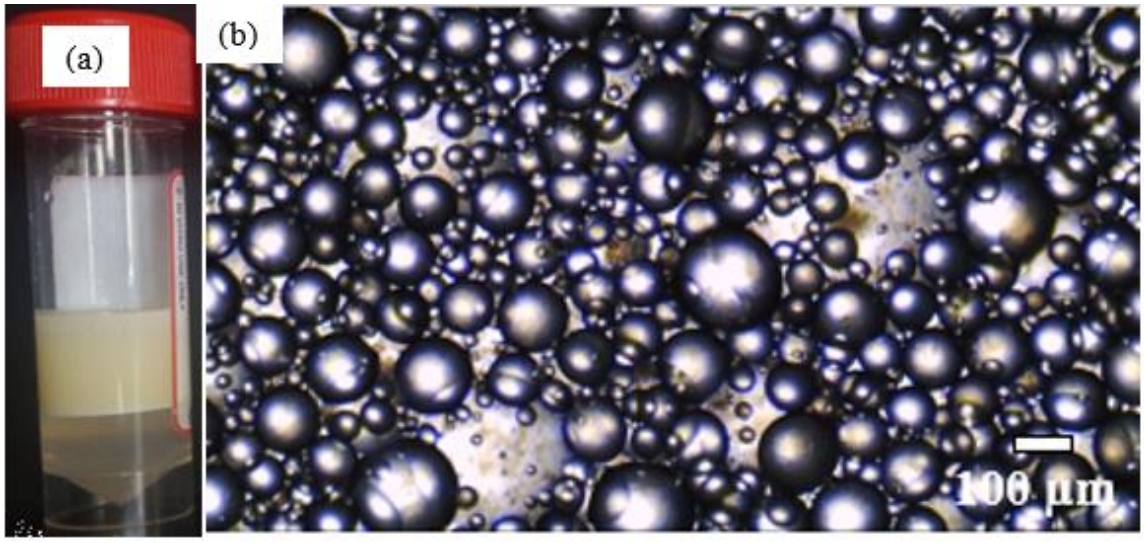
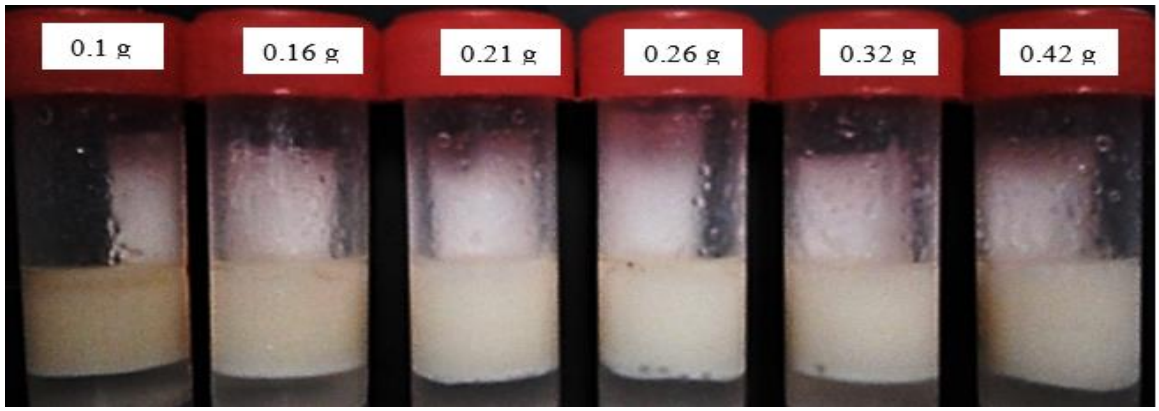


Fig. 3: (a) Photo after 24 hours of a vessel containing vegetable oil-water emulsion stabilized by 0.105g of soybean protein with $\phi_w = 0.5$. (b) Optical microscopic image showing the oil droplets in the vegetable oil-in-water emulsion shown in (a).

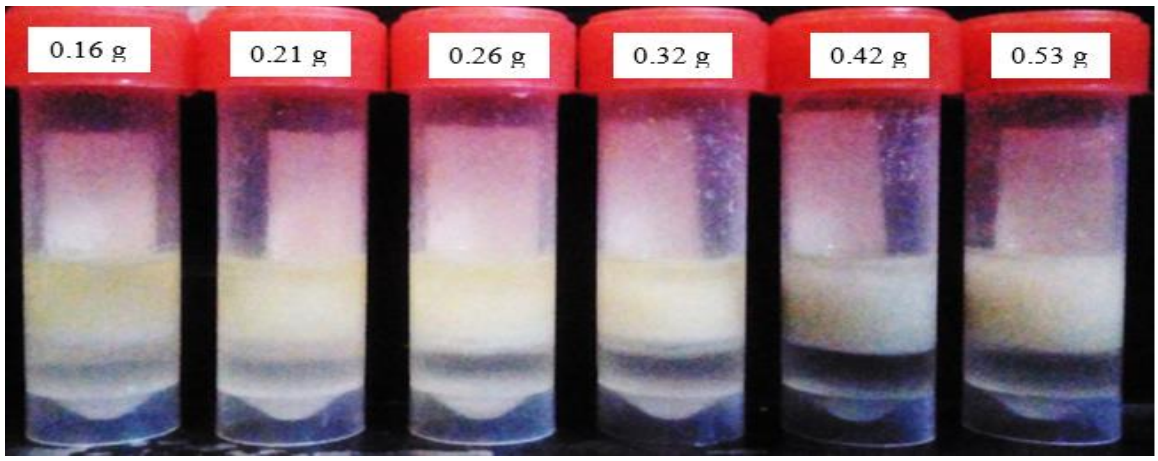
Emulsion prepared using soybean protein and microgel with the addition of salt

The effect of soybean protein and microgel concentration on the properties of vegetable oil-water emulsions is studied in this section. Emulsions were prepared at a volume fraction of water $\phi_w = 0.5$ as a function of emulsifier concentration with the addition of 0.0025 M NaCl. Emulsions are oil drops in water at all concentrations considered. Emulsions containing soybean protein are formed by vigorous handshaking for 2 min while emulsions stabilized by microgel are homogenized with a Hanchen Lab homogenizer with 12 mm head operating at 12, 000 rpm for 2 min. Fig 4 shows

the photographs taken after 2 hours of vessels containing vegetable oil-water emulsions stabilized by soybean protein and microgel at different emulsifier concentrations. For emulsions stabilized by soybean protein (Fig 4(a)), emulsions cream leaving an aqueous layer below. At all concentrations, no oil is seen above emulsion, indicating that all the systems are stable to coalescence. The stability of the emulsions is assessed after 7 days by measuring the amount of oil or water ejected. The fraction of oil or water resolved at this time is plotted as a function of concentration in Fig 5. The filled points represent the fraction of oil resolved via coalescence of o/w emulsions while the open points denote the fraction of aqueous phase resolved due to the creaming of the o/w emulsions. In both systems, the fraction of oil and water decreases with the concentration of emulsifier. The stability of the emulsion to coalescence increase with increasing the concentration of the soybean protein and microgel. The increase in the stability may be due to an increase in the number of emulsifiers at the interface which coat the drop surface [32]. The microscopic images of emulsions taken at different emulsifier concentrations are given in Fig 6. The oil droplets are spherical, discrete and polydisperse at all concentrations of soybean protein and microgel. The size of the oil drop size decrease upon increasing the concentration of both soybean protein and microgel. The variation of mean drop diameter with concentration is shown in Fig 7. Increasing emulsifier concentration has a significant influence on the emulsion drop size and the average droplet size decreases with emulsifier concentration up to a limit. This happens because more emulsifiers are available at the oil-water interfaces to stabilize smaller oil droplets and their accumulation at the interface protects the droplets against coalescence. The variation of mean drop size is consistent with the extent of coalescence of the oil drop.

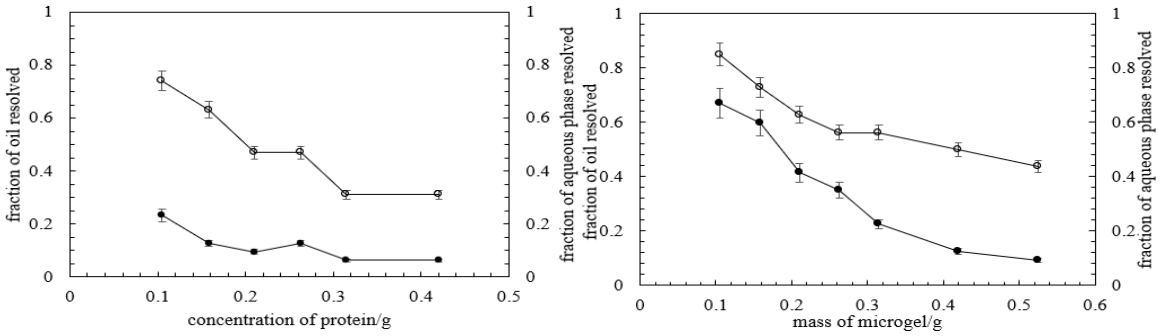


(a)



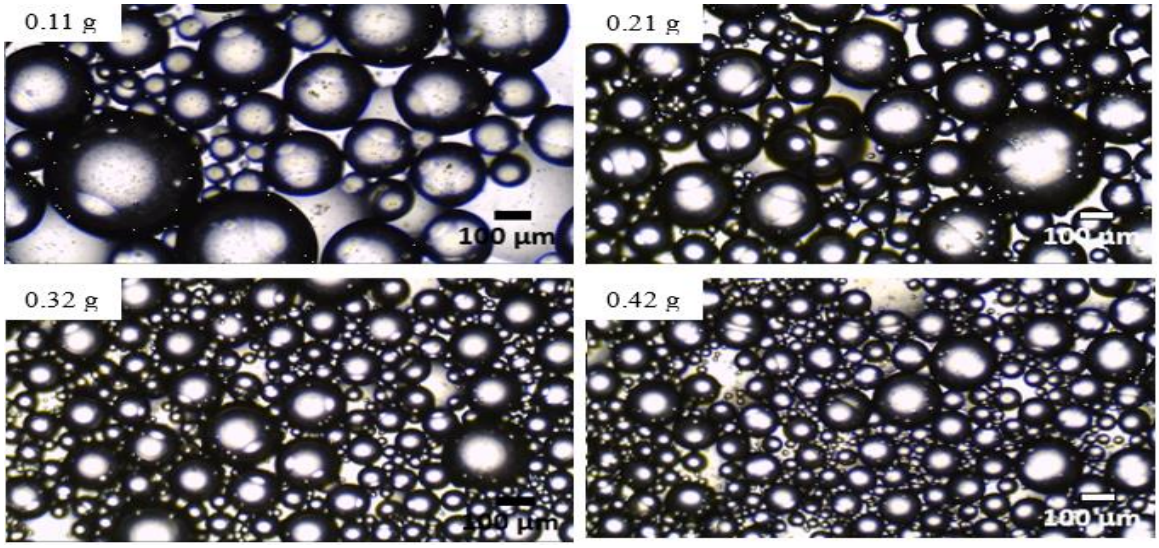
(b)

Fig 4: Photo after 2 hours of vessels containing vegetable oil-water emulsions stabilized by: (a) soybean protein and (b) microgel at different concentrations (given in g) with [NaCl] = 0.0025 M and ϕ_w = 0.5 .

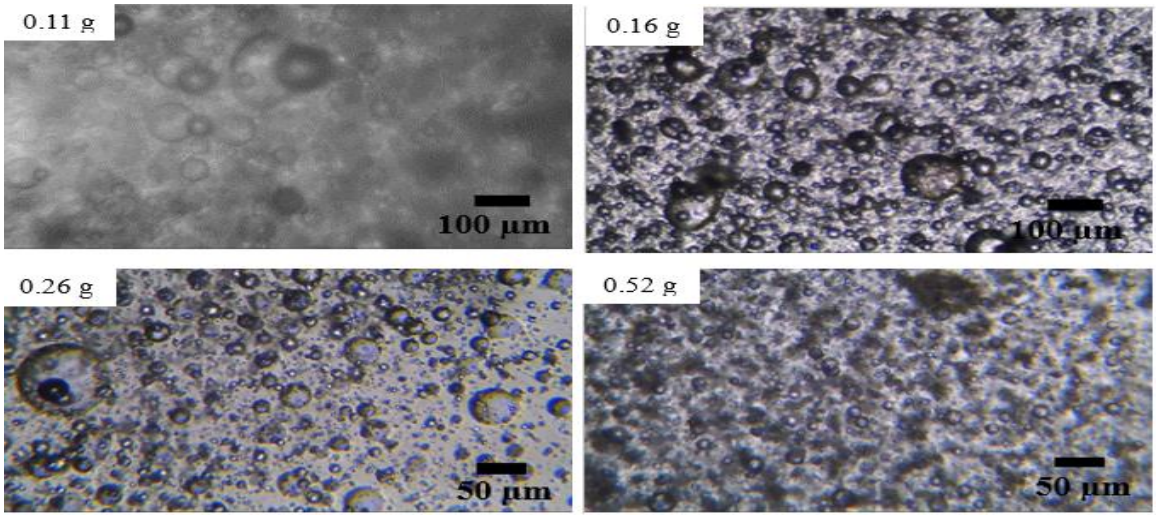


A B

Fig 5: Variation of fraction of oil (●) and aqueous phase (○) resolved after 7 days as a function of emulsifier concentration for emulsions stabilized by: (a) soybean protein (b) microgel.

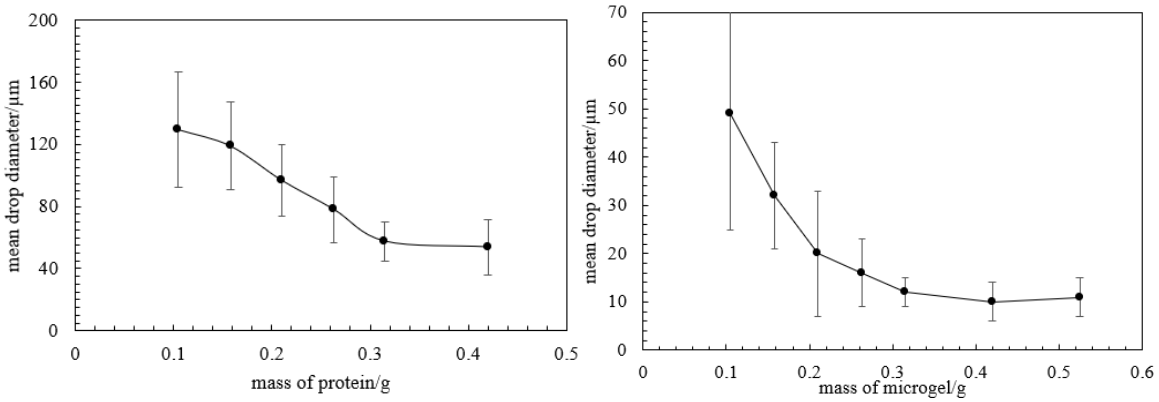


(a)



(b)

Fig. 6: Optical micrographs of vegetable oil-water emulsions taken immediately after preparation at different concentrations (given in g) with [NaCl] = 0.0025 M and ϕ_w = 0.5 for: (a) soybean protein and (b) microgel.



(a) (b)

Fig 7: Variation of the mean droplet diameter with emulsifier concentration for emulsions stabilized by (a) soybean protein and (b) microgel.

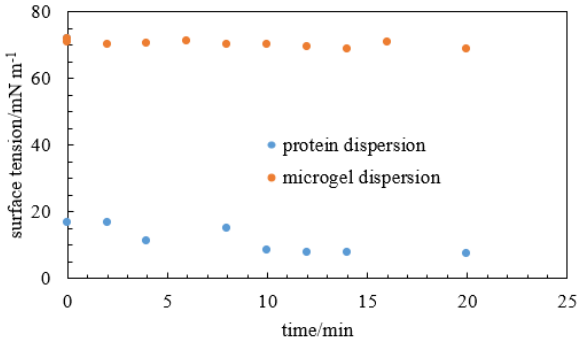


Fig 8: Variation of air-water surface tension with time for the dispersion containing 0.105 g of either soybean protein or microgel.

To understand the mechanism of stabilization by these emulsifiers, we have measured the surface tension of a dispersion containing 0.105 g of either soybean protein or microgel. The results are shown in Fig 8 for both systems. The surface tensions of pure water, pure vegetable oil and interfacial tension of vegetable oil and water are also measured and they are summarized in Table-2. As seen in Fig 8, the addition of soybean protein to water causes the surface tension to decrease significantly whereas the addition of microgel to water does not have a significant influence on the surface tension of water. The ability of a surface-active agent to aggregate at an interface usually favours the expansion of an interface. Given that π is the expanding pressure of the adsorbed layer of surfactant. The lowering of the surface/interfacial tension is given by [33]

$$\pi = \gamma_o - \gamma \qquad (6)$$

where is the surface tension of the pure liquid and π is the surface pressure. The reduction in surface tension (γ) is found to be 64.8 ± 0.1 mN m^{-1} and 1.9 ± 0.1 mN m^{-1} for the dispersion containing soybean protein and microgel respectively. For the system containing microgel, no significant lowering of surface tension was observed. This is consistent with the result reported by Palazolo *et al.* [34] for heat-treated soybean protein.

Table-2: Values of air-water (γ_{aw}) surface tension, air-oil (γ_{oa}) surface tension and oil-water (γ_{ow}) interfacial tension.

	γ_{aw}/mN m^{-1}	γ_{oa}/mN m^{-1}	γ_{ow}/mN m^{-1}
Surface tension	**71.9 ± 1.0**	**34.7 ± 0.6**	**22.1 ± 0.9**

For an emulsion stabilized by a solid particle, a simple mass balance equation based on geometrical considerations can be written, assuming complete adsorption of spherical particles at the interface. Drop size is related to the emulsifier concentration as follows [30, 35]

$$D = \frac{6\emptyset \times \Gamma}{(1-\emptyset)C} \qquad (7)$$

where c is the initial emulsifier concentration, ∅ is the volume fraction of oil, and D is the mean drop diameter. This equation predicts that the inverse of c should vary linearly with mean drop diameter for which can be obtained directly from the slope of D against For emulsion stabilized by the microgel, a linear relationship was obtained from the plot of D against while the graph for emulsion stabilized by soybean protein was non-linear. This observation shows that emulsions stabilized by microgel exhibit Pickering-like behaviour. The value of obtained for this system is approximately 70 mg m^{-2}. The result obtained from this analysis suggests that different

mechanisms of stabilization exist in these two systems. The stabilization mechanism in microgel-stabilized emulsion is based on the strategic adsorption of microgel at the interface. Thus, droplet surface is coated with microgel particles, forming a rigid support that prevents breaking of emulsion droplets. Our result here is similar to that reported by Liu and Tang [36]. They found that nanoparticles derived from soybean protein exhibited a good emulsifying ability and the properties of the resulting emulsions were akin to those of the conventional Pickering emulsions. Conversely, for emulsions stabilized by soybean protein, the addition of protein to the system lowers the interfacial tension and imparts needed stability to the system. The stabilizing action of the protein may be due to its amphiphilic nature. The reduction of surface/interfacial tension to a sufficiently low value usually enhances emulsification performance because only a relatively small increase in the surface free energy of the system is involved. From the thermodynamic point of view, the free energy change that accompanied the formation of an emulsion comes from two contributions. A surface energy term (ΔA) due to the large interfacial area which must be created and maintained between two immiscible liquids and a term due to the entropy of dispersion (since the production of many droplets is accompanied by an increase in configurational entropy, TΔS). From the second law of thermodynamics, free energy is given by [33, 37]

$$\Delta G = \Delta A\gamma - T\Delta S \quad (8)$$

In our system here, $\Delta A\gamma$ is likely to be less than $T\Delta S$, which means ΔG may be negative and the process is likely to occur. Thus, the formation of the emulsion may be favourable. This may account for the formation of the stable emulsion by handshaking the mixture of oil and water in the system containing soybean protein.

Emulsions prepared at high salt concentrations

In this section, the effect of NaCl concentration on the stability and type of emulsions stabilized by soybean protein is studied. Emulsions were prepared at a volume fraction of water $\phi_w = 0.5$ as a function of NaCl concentration. Emulsions were made by handshaking the mixture of oil and water for 2 min. Emulsions are oil-in-water at all concentrations of NaCl considered. Fig 9 shows the photograph taken after 24 hours of vessels containing vegetable oil-water emulsions stabilized by soybean protein at different NaCl concentrations. As seen in Fig 9, emulsions creamed leaving below the varying amount of aqueous phase depending on the salt concentration. At all concentrations of salt, no oil was seen above emulsion, indicating that all the systems were stable to coalescence. The stability of the emulsions was assessed after 2 months by measuring the amount of oil or water ejected. The fraction of oil or water resolved at this time was plotted as a function of salt concentration in Fig 10. At all salt concentrations, little or no oil was resolved and the fraction of aqueous phase resolved was almost the same at all salt concentrations of NaCl considered. The microscopic images of emulsions taken at different salt concentrations are given in Fig 11. The oil droplets are spherical, discrete and polydisperse at all salt concentrations. The variation of mean drop size with NaCl concentration is shown in Fig 12. Increasing salt concentration does not have a significant influence on the emulsion drop size.

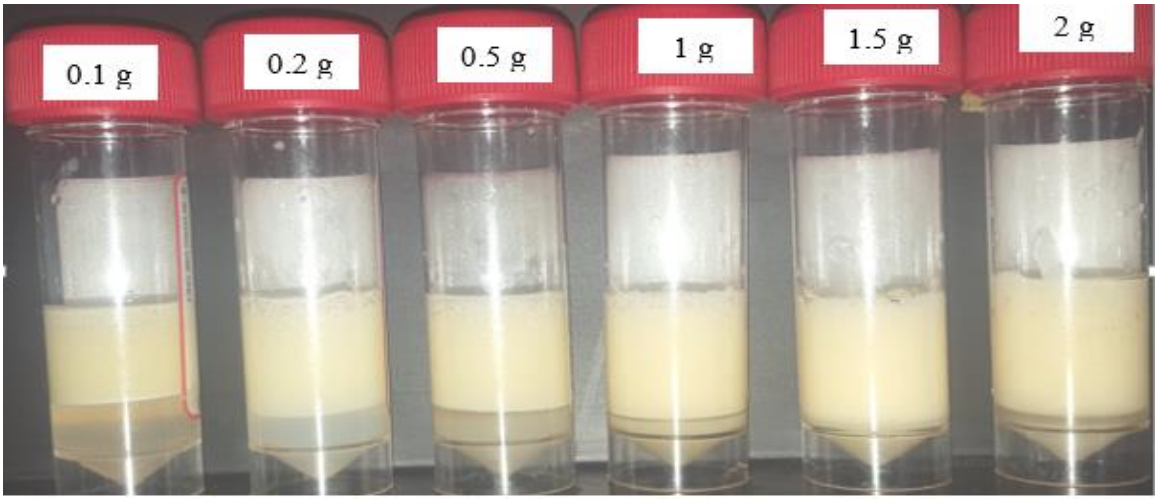


Fig 9: Photo after 24 hours of vessels containing vegetable oil-water emulsions ($\phi_w = 0.5$) prepared at different salt concentrations (given) stabilized with 0.52 g of soybean protein at pH 4.5.

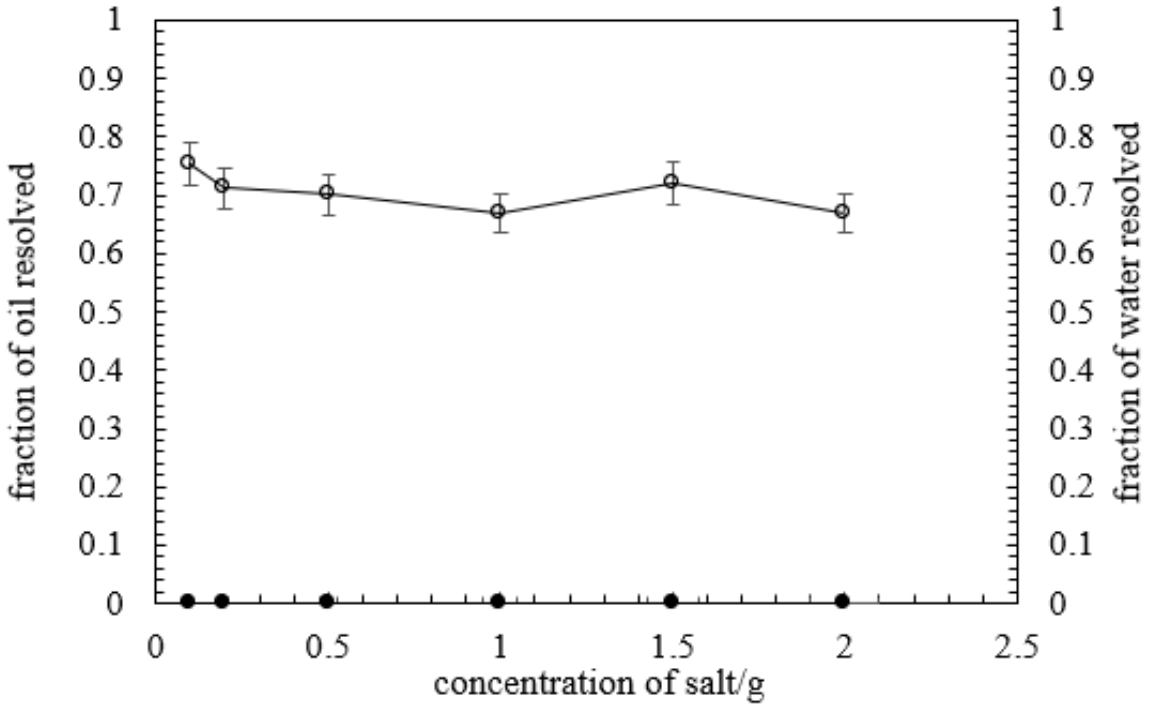


Fig. 10: Fraction of oil phase (●) and aqueous (○) phase resolved after 2 months from vegetable oil-water emulsions (ϕ_w = 0.5) at different salt concentrations.

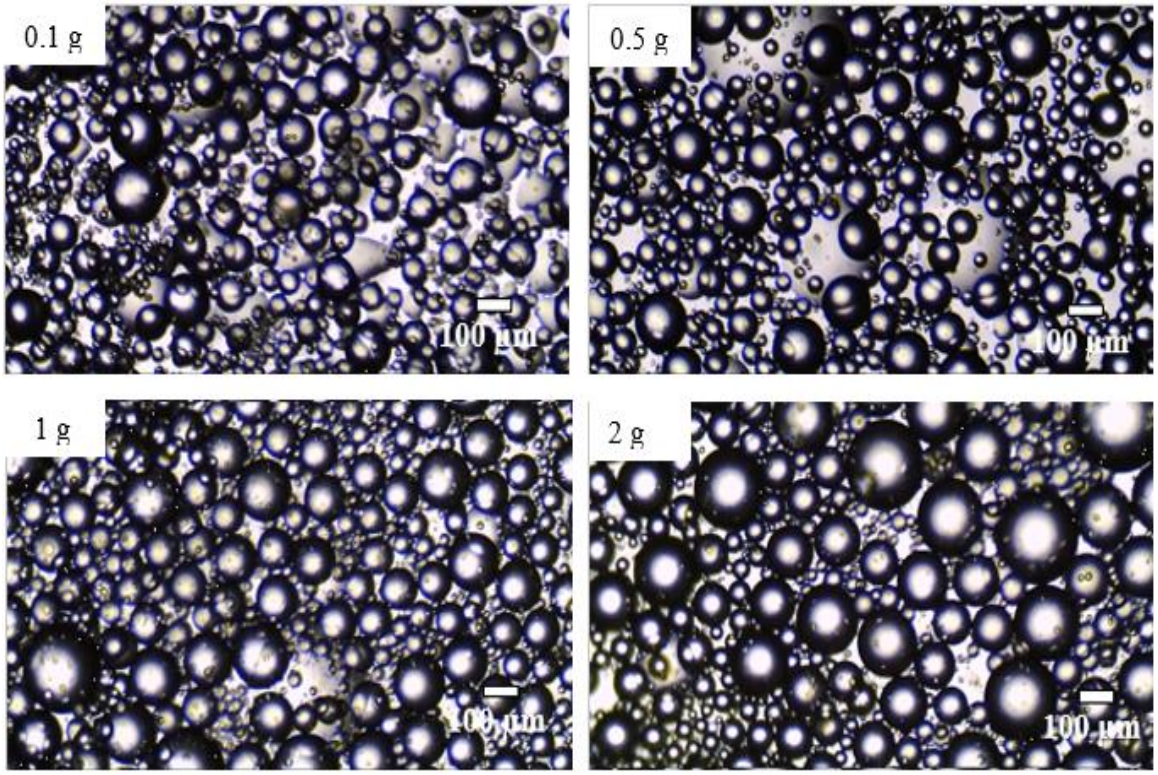


Fig. 11: Optical microscopic images of o/w emulsions stabilized by soybean protein at different NaCl concentrations.

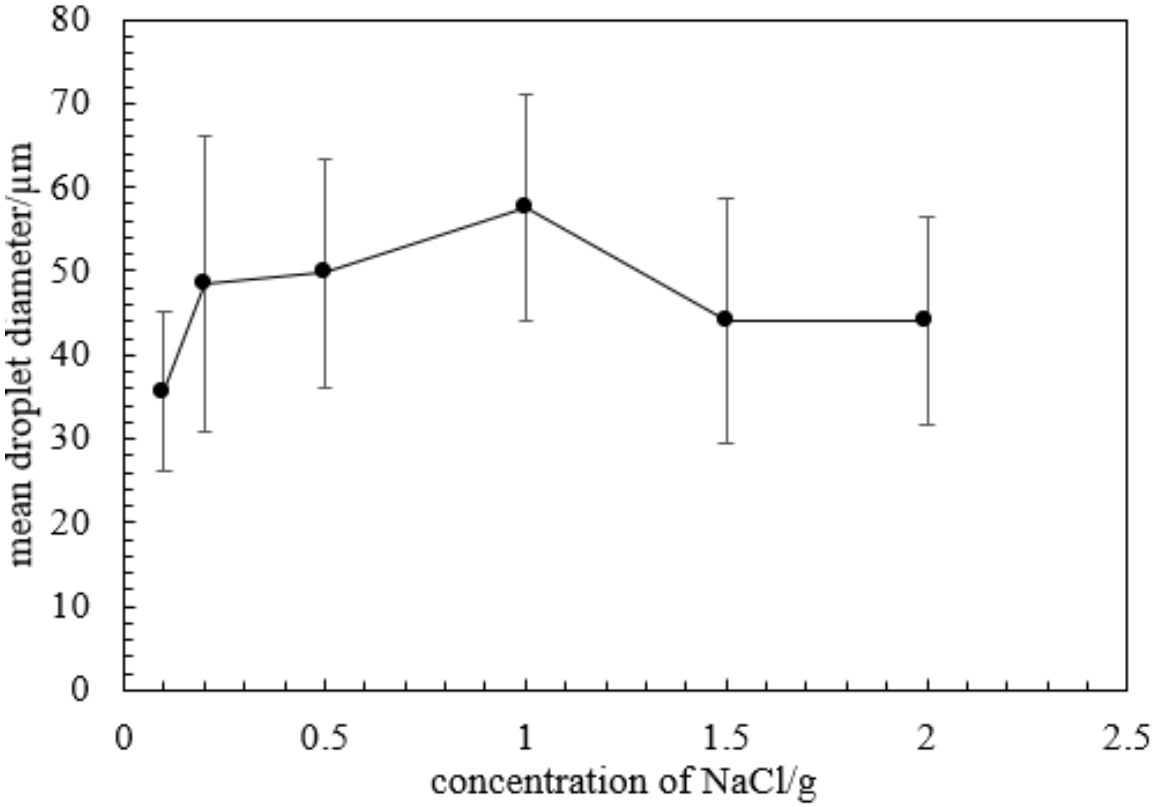


Fig. 12: Variation of mean drop diameter as a function of salt concentration for vegetable oil-water emulsions (ϕ_w = 0.5) stabilized by soybean protein.

Conclusion

The stability of o/w emulsions stabilized by soybean protein and microgel is investigated. The protein was obtained from *n*-hexane defatted soybean flour. Emulsions stabilized by protein without salt breakdown after 3 days of storage due to decomposition of the protein whereas emulsion stabilized by microgel phase-separated after 1 hour of preparation. The effect of emulsifier concentration on the stability and drop size of emulsion was studied. For both systems, increasing the concentration of emulsifier improve the stability of the emulsion with a corresponding decrease in the size of emulsion droplet. Our results show that the mechanism of stabilization of emulsions stabilized by soybean protein and microgel is not the same. The mechanism of emulsion stabilization by the protein is by lowering of interfacial tension whereas in emulsion stabilized by microgel is based on strategic adsorption of microgel at the interface. Increasing the concentration of NaCl improves the stability of emulsion stabilized by the protein but does not affect the drop size.

Conflicts of interest

The authors declare no competing financial interest.

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