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Effect of protein and oil concentration on the emulsion stability of soy protein isolate

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Abstrat : The emulsion stabilizing properties of soy protein isolate (SPI) were investigated in terms of the protein and oil concentration. Particularly, the dependence of emulsion stability on the oil particle size and viscosity of emulsion was studied in conjunction with the adsorption pattern of protein onto the water/oil interface during emulsification. The data showed that increasing protein concentration decreased the oil particle size and increased the emulsion viscosity, resulting in the enhanced emulsion stability. In contrast, increasing oil concentration increased both the oil praticle size and the emulsion viscosity, and thus emulsion stability varied depending on which factor predominated the overall emulsion system(Received August 24, 1992, accepted October 22, 1992).

Emulsion is defined as an intimate mixture of two immiscible liquids, one being dispersed in the other in the form of fine droplets.[1)] Emulsions in foods consist of two distinct phases, i.e., water and oil, where emusifiers are sited at the oil/water interface.[2,3)] Proteins, naturally occurring emulsifiers, aid in the formation of emulsions by lowering interfacial tension and stabilize the emulsions once formed by providing the reduced interfacial tension, the electrostatic repulsion and the protective rigid film encapsulating the oil droplets.[4-6)] The capability of proteins for forming and stabilizing emulsions is of primary significance in food processing, and thus proteins are widely employed as emulsifiers to a variety of emulsion foods such as butter, margarine, salad dressing, ice cream, whipped toppings, sauage, comminuted meat, coffee whitener, mayonnaise and etc.[3,7,8)]

Emulsions are thermodynamically unstable and thus are destroyed to some extent as time goes, where creaming and coalescence act as the principle mechanism.[9-11)] Emulsion stability (ES) represents the extent to maintain the initial emulsion system under the defined conditions. It has been well documented that the emulsion viscosity and oil particle size are the most important two parameters determining ES, and creaming and coalescence are inhibited by decreasing oil particle size and increasing emulsion viscosity.[12-14)]

In this research the stability of emulsions prepared by soy protein isolate (SPI) was investigated in terms of protein and oil concentration with particular reference to the oil particle size and emulsion viscosity. In addition, we also report the adsorption pattern of SPI during emulsification as a function of the protein and oil concentration.

Materials and Methods

Materials

Defatted soy flour (Cheil Sugar Co., Korea) was used to extract soy protein isolate (SPI) by isoelec-

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tric point (pH 4.5) precipitation. The detailed procedure for this was described previously by Hwang *et al.*[15] The extracted SPI was neutralized to pH 7.0 and freeze-dried. The protein content of resulting SPI was determined to be 91.0% by the Lowry method.[16] Soybean oil (Cheil Sugar Co., Korea) constituted the oil phase of emulsions.

Preparation of oil-in-water (o/w) emulsions

Appropriate amount of SPI was dissolved in distilled water using a magnetic stirrer for 1 hr at room temperature, in which soybean oil was added. Then, the mixture was homogenized for 5 min at 7,000 rpm by using Sorvall Omnimixer (DuPont Instrument Co., Wilmington, DE, USA; Model 17105) in a 30℃ waterbath.

Determination of emulsion stability

Emulsion stability (ES) was evaluated by the method of Tornberg and Hermansson[17] with slight modification. The emulsion samples placed in 20×110 mm test tubes were stored for 2 hrs in a 30℃ incubator, after which the oil content of 15 m*l* emulsions from the tube bottom was measured by the Gerber method.[18] Then, ES was calculated as follows:

$$ES = F/F_o \times 100 \ (\%)$$

where F_o is the initial oil content of emulsions, and F is the oil content of the lower layer after 2 hrs storage at 30℃. The higher value of ES indicates the more stable emulsions.

Measurement of oil particle size

The oil particle size of emulsion was determined by the microscopic method. The emulsions were diluted 50 times by 50% (v/v) glycerine and transferred to the slide glass. Then, the average oil particle size of emulsion was obtained from approximately 200 oil particles present in 5 different emulsion regions.

Measurement of emulsion viscosity

Viscosity measurements of emulsions were performed with Haake Rotoviscometer (Model R-12) at 25℃. In this research, the apparent viscosity, expressed by centipoise, was determined at 1385 sec^{-1} of shear rate after the emulsions were loaded for 1 min.

Measurement of SPI adsorbed onto the oil/water interface

The amount of protein adsorbed onto the water/oil interface during emulsification was measured by the method of Tornberg[19] with modification. The majority of oil phase in emulsion was removed by centrifuging at 3,000×g for 20 min, and the rest emulsion was diluted 20~120 times depending on the protein concentration. The diluted emulsion was filtered through Whatman filter paper (pore size 0.45 μm, diameter 2.5 cm) to eliminate the remaining oil particles. Then, the protein content of the filtrate was measured by the method of Lowry *et al.*[16], where bovine serum albumin (BSA) was utilized for preparing the standard.

The absorption ratio (AR) was obtained as follows:

$$AR = (P_o - P)/P_o \times 100 \ (\%)$$

where P_o is the initial protein concentration, and P is the protein concentration after centrifugation and filtration. The protein content adsorbed onto the water/oil interface during emulsification is also presented as mg protein per m*l* oil, which wil be referred to as absorbed protein (AP) in the text. In addition, the total surface area (TSA) of the oil particles per unit volume of oil formed during emulsification is equal to the surface area per a particle times the number of particles per unit volume as follows[20]:

$$\frac{\text{total surface area}}{\text{m}l\ \text{oil}} = \frac{\text{surface area}}{\text{a particle}} \times \frac{\text{number of particles}}{\text{m}l\ \text{oil}} = (4\pi r^2) \times \frac{1}{(4/3)\pi r^3} = \frac{3\ (\text{cm}^2)}{r\ (\text{cm})} = \frac{3 \times 10^{20}\ (\text{Å}^2)}{r\ (\mu\text{m})}$$

where A is the angstrom units, and r is the radius of an oil particle.

Results and Discussion

Fig. 1 shows the relations of emulsion stability (ES), average oil particle size and emulsion viscosity as a function of protein concentration. As the protein concentration increased, the average oil particle size decreased, whereas the emulsion viscosity increased. It is evident that the possibility of collisions with oil particles during emulsion formation increases with protein concentration, which correspondingly reduces the oil particle size leading to the increased viscosity at the same amount of oil phase.[21] In reality, both the decreased oil particle size and the increased viscosity favor the stability of emulsion system. Accordingly, it is apparent that ES increased with increasing protein concentration. The same positive effect of protein concentration on ES was also reported for other protein sources.[22-24] In contrast, Hwang *et al.*[15] demonstrated that ES was not proportional to the protein concentration in the presence of other emulsifiers such as monoglyceride due to their mutual competition for oil. In this case, finding the optimum HLB (hydrophilic-lipophilic-balance) was of primary importance for the maximum ES.[15]

The absorption ratio (AR), absorbed protein (AP) and total surface area (TSA) are given in Table 1 as a function of protein concentration. AR increased up to 2.0% protein concentration, above which it decreased. In contrast, AP exhibited the increasing trend up to 3.0% concentration, which is attributable to the reduced particle size and the corresponding increased TSA at the same oil concentration with incresing protein concentration. This is in good agreement with the results for whey proteins.[25]

The effect of oil concentration on ES, oil particle size and viscosity is presented in Fig. 2. It can be seen that both the oil particle size and the viscosity increased together with oil concentration. Increasing oil concentration relatively reduced the protein content which could be adsorbed onto the interface

Table 2. Absorption ratio (AR), absorbed protein (AP) and total surface area (TSA) as a function of oil concentration at 1.0% SPI concentration

Oil concentration (%)	AR (%)	AP (mg protein/ml oil)	TSA×10^{-20} (Å^2/ml oil)
5.0	8.4	14.7	0.97
10.0	10.5	8.7	0.81
20.0	14.0	5.2	0.55
30.0	16.4	3.5	0.42

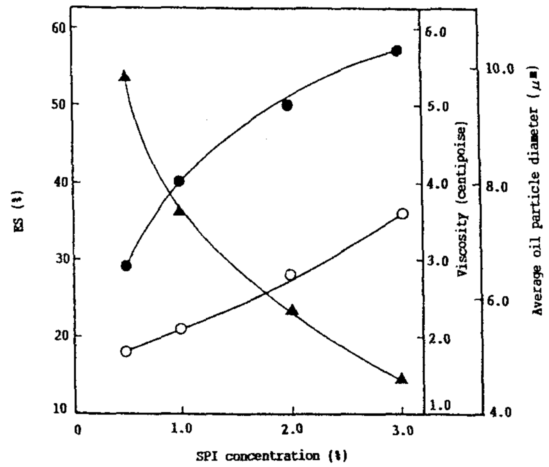

Fig. 1. Effect of soy protein isolate (SPI) concentration on emulsion stability (●—●), average oil particle diameter (▲—▲) and viscosity (○—○) at 10% oil concentrtion.

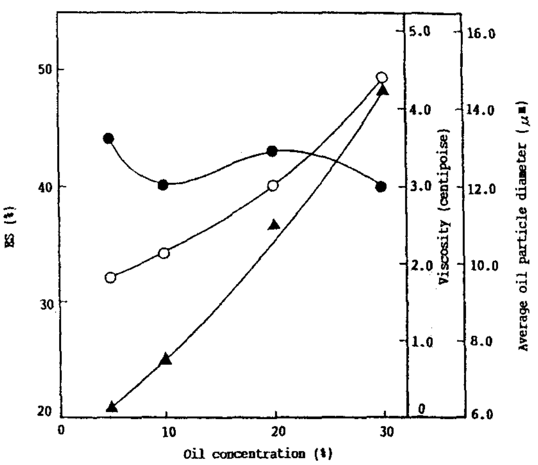

Fig. 2. Effect of oil concentration on emulsion stability (●—●), average oil particle diameter (▲—▲) and viscosity (○—○) at 1.0% SPI concentration.

per unit oil volume during emulsification, resulting in the increased particle size. On the other hand, the increased viscosity can be ascribed to the increased internal phase with increasing oil concentration. This result was supported by the report of Yamauchi *et al.*[25] Here, it should be noted that simultaneous increases in the particle size and viscosity confer the anti-complementary effect on ES. In this case, ES is dependent on which factor controls the whole emulsion system. For example, the highest ES was obtained at 5% oil content despite the lowest emulsion viscosity. This is because the lowest oil particle size at 5% oil concentration acted as the predominant factor for ES. In contrast, at 30% oil concentration having the highest emulsion viscosity, ES was relatively low due to the large particle size. These results are not consistent with the report of Acton and Saffle,[26] showing that increasing oil concentration resulted in enhancing ES. This may indicate that they employed the emusion system in which the emulsion viscosity controlledd ES more efficiently than the oil particle size. On the other hand, Yamauchi *et al.*[25] reported that ES was propotional to the oil concentration at high protein concentration, but not at low protein concentration. This suggests that the effect of oil concentration may vary depending on the protein concentration.

Table 2 shows AR, AP and TSA during emulsification as a function of oil concentration. It can be seen that AR increased with the oil concentration. This is because the possibility of protein to contact with oil increases with the oil concentration. As a result, the total protein involved with emulsion formation, representing AR, increased with oil concentration. *In cotrast,* both AP and TSA decreased at the same condition. This can be interpreted that the oil particle size increased with increasing oil concentration, and those two factors quantitated on the basis of per m*l* oil decreased correspondingly.

Table 1. Absorption ratio (AR), absorbed protein (AP) and total surface area (TSA) as a function of SPI concentration at 10% oil concentration

SPI concentration (%)	AR (%)	AP (mg protein/m*l* oil)	TSA$\times 10^{-20}$ (Å^2 /m*l* oil)
0.5	9.4	3.9	0.61
1.0	10.5	8.7	0.81
2.0	10.7	17.7	1.03
3.0	9.9	24.6	1.36

Conclusions

Tornberg[20] stated that emulsification involved disruption and coalescence as dynamic process, and the formation of emulsions was dependent on the number of collisions of protein molecules with oil particles. Consequently, the oil and protein concentration are the key factors affecting the oil particle size and the emulsion viscosity during emulsification. In general, the smaller the oil particle size and the higher the emulsion viscosity the more stable the emulsions. In some cases, these two factors are anti-complementary as shown in this research for the effect oil concentration, and thus they should be taken into account together to clearly understand and predict emusion stability.

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단백질과 기름농도가 분리대두단백질의 유화안정성에 미치는 영향

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초록 : 본 연구에서는 단백질과 기름의 농도가 분리대두단백질의 유화안정성에 미치는 영향을 연구하였다. 특히 유화안정성과 에멀젼의 기름입자크기 및 점도와의 상관관계를 구명하였으며, 또한 유화과정 중 단백질이 물과 기름 사이의 계면에 흡착하는 현상을 조사하였다. 단백질의 농도가 증가할수록 기름입자의 크기는 감소하고 점도는 증가하여 결과적으로 유화안정성이 증가하였다. 반면에 기름농도가 증가한 경우 기름입자의 크기와 점도가 모두 증가하였으며, 이 경우에는 두 요인의 상대적인 지배정도에 따라 에멀젼의 유화안정성이 결정되었다.