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Effect of phosphate salts on the emulsion stability of soy protein isolate

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Abstract : A study was conduted to investigate the effects of phosphate salts (Na_2HPO_4 and K_2HPO_4) on the emulsion stability of soy protein isolate (SPI) in terms of the salts concentration and addition order. When phosphates were added before emulsification, emulsion stability (ES) of SPI was improved at the concentration of 10 mM, while ES was decreased by addition of phosphates after emulsification. At high phosphate concentrations, ES of SPI was decreased by the addition of phosphates, regardless of the addition order. ES of SPI at the isoelectric point (pH 4.5) or in the presence of $CaCl_2$ was greatly enhanced by the phosphates. In both cases, the overall ES profile was found to be nearly similar to the solubility profile of SPI, indicating the positive relationship between solutibility and emulsion stability of SPI(Received March 14, 1992, accepted April 24, 1992).

Phosphates are widely used in food systems because of their versatile functionalities as buffers, acidulants, water binding agents, leavening agents, dispersants, emulsifiers and sequestrants related to antioxidative and antimicrobial properties.[1-4] The beneficial effects of phosphates to semi-solid emulsion foods such as processed meats and cheeses have been well documented to date, where the phosphates enhance the emulsion stability by affecting the solubility and conformation of proteins.[5-11] The details regarding the chemistry and functional role of phosphates can be found elsewhere.[12-19]

However, few studies have been investigated on the phosphate effects as emulsifiers in the liquid emulsion system of proteins. In general, the functionalities of proteins are significantly restricted at the isoelectric point or in the presence of multivalent ions, because these conditions inhibt the solubilization of proteins. In this research, soy protein isolate (SPI) is chosen as a protein source for emulsification, because it is one of the typical plant proteins frequently employed in liquid emulsion foods. This research aims at investigating the influence of phosphates (Na_2HPO_4 and K_2HPO_4) on the emulsion stabilizing properties of SPI in terms of the concentration and addition order. The phosphate effects were also investigated at the isoelectric point (pH 4.5) of SPI or in the presence of calcium salts ($CaCl_2$) with particular reference to the solubility.

Materials and Methods

Materials

Soy protein isolate (SPI) was extracted from defatted soy flour (Cheil Sugar Co., Korea) by isoelectric point (pH 4.5) precipitation as described previously by Hwang *et al.*[20] The extracted SPI was neutralized to pH 7.0 and freeze-dried. The resulting SPI showed approximately 91.0% of the protein

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content by the Lowry method.[21] Soybean oil (Cheil Sugar Co., Korea) was used as an oil phase in emulsions. The phosphate salts, i.e., Na_2HPO_4 and K_2HPO_4, were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Determination of protein solubility

Protein solutions (1% w/v) were prepared by dissolving 1g of SPI in 100 m*l* of distilled water for 1 hr at room temperature, in which phosphate salts were added in the concentration range of 0~40 mM. The protein solutions with or without salts were centrifuged at 22,000×g for 10 min, and then the protein concentration of supernatant was determined by the Lowry method[21] using boving serum albumin as a standard. The protein solubility was expressed as the ratio (%) of the protein concentration of supernatant to that of original protein solution.

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

The aqueous phase of emulsion was prepared by dissolving 0.9g of SPI in 90 m*l* of distilled water, in which 10 m*l* of soybean oil was added as an oil phase. Then, the mixture was homogenized for 5 min at 7,000 rpm by using Sorvall Omnimixer (DuPont Instrument Co., Wilmington, DE; Model 171 05) in a 30 ℃ waterbath. All salts were added to the protein solutions before emulsification or after emulsification and mixed thoroughly with using magnetic stirrer.

Measurement of emulsion stability

Emulsion stability (ES) was evaluated by the method of Tornberg and Hermansson[22] 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.[23] 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.

Resullts and Discussion

Fig. 1 shows the effects of phosphate salts, i.e., Na_2HPO_4 and K_2HPO_4, on emulsion stability (ES) of SPI with respect to the concentration and addition order. When phosphates were added before emulsification, the emulsions were increasingly stabilized, showing the maximum at 10 mM concentration. However, approximately above 20 mM, ES was lower than those generated without phosphates. In contrast, when phosphates were added after emulsification, ES showed lower values than those of emulsions without phosphates, and as the phosphate concentration increased, ES decreased further. The anionic phosphates can bind with the positively charged groups of proteins, which results in crosslinking the protein molecules, especially at the high phosphate concentrations.[2,11] Likewise, the phosphates may act to crosslink the proteins surrounding the oil particles in the emulsion. This will facilitate the flocculation of the oil particles and

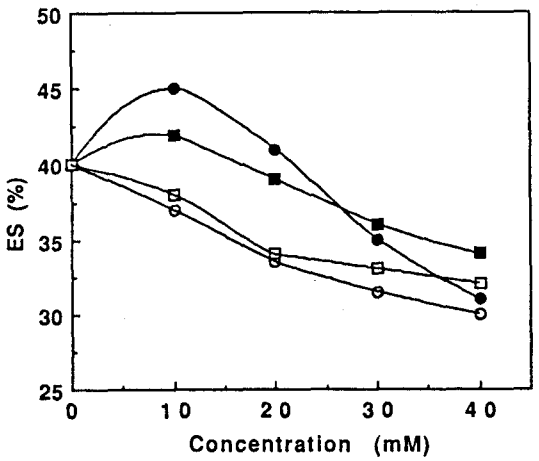

Fig. 1. Effects of phosphate concentration and addition order on emulsion stability.
The addition order of phosphates was as follows : Na_2HPO_4, ●—● : Before emulsification, ○—○ : After emulsification; K_2HPO_4, ■—■ : Before emulsification, □—□ : After emulsification

the subsequent coalescence and creaming of emulsions. From this view, it is interesting to note that phosphates enhance the emulsion stabilizing properties of SPI at low concentrations when added before emulsification, as shown in Fig. 1. Ellinger[14] reported that sodium and potassium diphosphates solubilized proteins and therefore formed the protective films around the fat globules in order to improve emulsification. This argument is also agreed with the report of Halliday.[1] Accordingly, the results shown in Fig. 1 can be interpreted that, when the phosphates were added before emulsification, they contribute to the solubility or dispersability of SPI at low phosphate concentrations. Both Na_2HPO_4 and K_2HPO_4 showed the same ES trends regardless of the salt type of phosphates, although the magnitude of ES was somewhat different. Besides phosphates, sodium citrate also exhibited the similar results as phosphate salts with regard to the concentration and addition order.[24]

Significant reduction in solubility of SPI in the acidic region limits its utilization in foods. Aoki *et al.*[25,26] reported that SPI showed the lowest solubility and correspondingly the lowest ES at the isoelectric point (pH 4.5). In this research, the effects of phosphates on ES of SPI at pH 4.5 was investigated as a function of phosphate concentration. As shown in Fig. 2, ES was greatly increased by the addition of 5 mM phosphates and reached the maximum ES at 10 mM, above which ES however was gradually reduced. It is important to note that ES pattern is nearly similar to solubility pattern.

Calcium salts are inherently present in foods, or alternatively may be fortified to foods containing proteins. In these cases the calcium ions can form salt bridges with protein molecules,[27] and thus the functional properties of proteins are significantly decreased due to the reduced solubility. Fig. 3 shows the effects of $CaCl_2$ concentration on ES of SPI. The addition of $CaCl_2$ after emulsification resulted the higher ES than that before emulsification in the $CaCl_2$ concentration ranging from 0~3 mM. In the case of $CaCl_2$ addition after emulsification, SPI can be used as an emulsifier at least during emulsification, although the emulsions are inevitably affected by calcium. In contrast, when added before emulsification, the calcium ions can crosslink the SPI molecules. Therefore, the emulsifying capability of SPI is restricted during emulsification by the low solubility. However, in both cases ES of SPI became negligible above 3 mM $CaCl_2$ concentration. In this research we investigated the phosphate effects on ES of SPI in the presence of 5 mM $CaCl_2$ imparting the negligible ES. Fig. 4 shows that ES was significantly increased up to 20 mM concentration in the presence of 5 mM of $CaCl_2$. This may be attributable to the sequestrant function of phosphates for calcium ions.[4,14] The phosphate-calcium complex is stable and soluble, which can be absorbed through the intestine and utilized by the

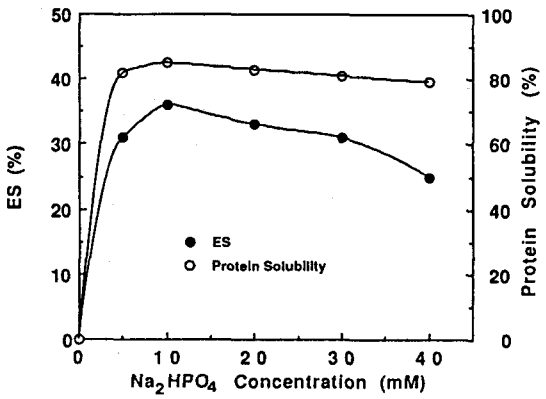

Fig. 2. Emulsion stability and protein solubility as a function of Na_2HPO_4 concentration at pH 4.5.

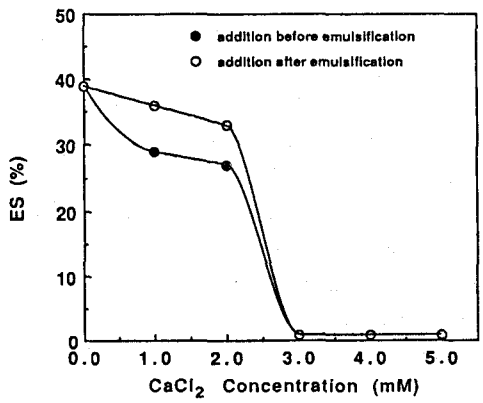

Fig. 3. Effects of $CaCl_2$ concentration and addition order on emulsion stability.

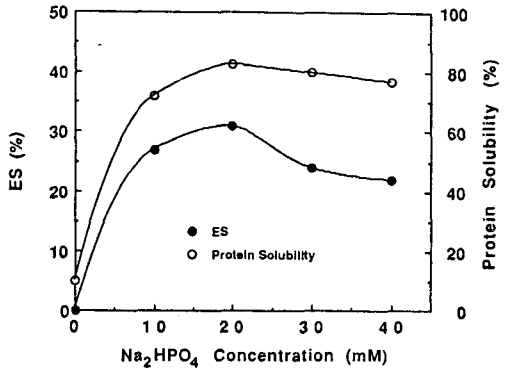

Fig. 4. Emulsion stability and protein solubility as a function of Na_2HPO_4 concentration in the presence of 5 mM $CaCl_2$.

body.[14] Morever, the absorption and retention of calcium ions may be increased in the form of complexes with phosphates.[28] Hwang[24] also reported that the phosphate addition before $CaCl_2$ as shown in Fig. 4 resulted in the higher ES than the opposite order. It should be noted the ES profile is similar to the solubility profile, indicating the significance of solubility to the emulsion stabilizing properties of SPI.

Conclusions

The stability of emulsions generated by SPI were significantly dependent on the concentration and addition order of phosphates. Particularly, the phosphate effects were pronounced at the isoelectric point (pH 4.5) of SPI or in the presence o calcium ions. In these cases, ES were closely related to the protein solubilizing capability of phosphates. The positive contribution of solubility to ES of SPI was also reported elsewhere.[29-31] Therefore, it is anticipated that phosphates can be successfully employed to improve ES of proteins under the unfavorable conditions for the functionalities of proteins, i.e., at the isoelectric point or in the presence of cations such as calcium, copper or magnesium. The optimum phosphate concentration should be found to meet the highest ES in combination with the appropriate addition order, depending on individual emulsion systems. In this research, we employed only two phosphate salts, i.e., Na_2HPO_4 and K_2HPO_4, and thus further researches are suggested to find the applicability of other phosphate types to the variety of liquid emulsions.

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분리 대두 단백질의 유화 안정성에 관한 인산염의 영향

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초록 : 분리 대두 단백질의 유화 안정성에 관한 인산 염(Na_2HPO_4, K_2HPO_4)의 농도와 첨가 방법의 영향을 연구 하였다. 유화 과정 이전에 인산 염을 첨가하였을 경우 10 mM의 농도에서 유화 안정성이 증가하였으나, 유화 과정 이후에 첨가한 경우에는 유화 안정성이 감소하였다. 반면에 높은 인산 염 농도에서는 첨가 방법에 관계없이 유화 안정성이 감소하였다. 한편 분리 대두 단백질의 등전점(pH 4.5)이나 $CaCl_2$의 존재 하에서 인산 염을 첨가한 경우 유화 안정성은 크게 증가 하였으며, 이 때 전체적인 유화 안정성은 분리 대두 단백질의 용해도와 유사한 경향을 보였다. 이는 분리 대두 단백질의 유화 안정성은 그 용해도와 밀접한 관계가 있다는 것을 의미한다.