Emulsifying Properties of Corn Germ Proteins[1]

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ABSTRACT

Emulsifying capacity and emulsion stability of hexane-defatted corn germ protein obtained by modified and conventional extraction processes were studied. Defatted corn germ protein obtained by the modified extraction process had higher emulsifying capacity and emulsion stability than that obtained by the conventional process. The highest emulsion stability was obtained with 7% defatted corn germ protein and 40% fat content; 70.39% water was retained by the emulsion. High emulsion stability was obtained as the result of specific protein properties. Increasing fat content in emulsions from 20 to 40% had a positive effect on emulsion stability. Because of its better functional properties, hexane-defatted corn germ protein obtained by the modified process is recommended for utilization as an additive in food products.

Emulsifying capacity and emulsion stability of defatted corn germ protein can be used to define how these proteins can be added to existing foods and how they can replace more expensive proteins traditionally used. A knowledge of the emulsifying properties of defatted corn germ protein is necessary to evaluate their potential use as food additives.

The stabilizing effect of proteins in emulsions results from the protective barrier they form around fat droplets, which further prevents their coalescence (Kinsella 1979). Long-term stability of emulsions depends basically on the thickness and strength of adsorbed protein films at the oil-water interface.

Emulsifying capacity and emulsifying stability are important functional properties of food proteins. Many chemical and physical factors are involved in the formation, stability, and textural properties of protein-fat-water emulsions. Emulsifying capacity and emulsion stability depend on the properties of the stabilizer and vary with the type of protein, its concentration, pH, ionic strength, and viscosity of the system. Nakai (1980) reported that solubility, surface hydrophobicity, and molecular flexibility influence the emulsification behavior of globular proteins.

Crenwelge et al (1974), working with soybean protein, found a direct correlation between solubility of the protein suspension and emulsifying capacity of proteins. At the same time, some investigators have suggested that solubility is not necessarily associated with emulsifying capacity and emulsion stability of vegetable proteins (Aoki et al 1980, McWatters and Holmes 1979a). McWatters and Holmes (1979b) and Ramanatham et al (1978) showed that emulsifying capacity of peanut protein cannot be predicted solely on the basis of protein solubility level. Solubility appears to contribute more to the quality of emulsions formed than to quantities of oil emulsified.

Some proteins have optimal emulsifying properties at their isoelectric points (egg white, gelatin), whereas others perform best at pH values away from their isoelectric points (soybean and peanut proteins). The pH influences the emulsifying capacity of proteins indirectly by affecting their solubility. A number of studies have shown that the pH-emulsifying property profile of various proteins resembles the pH-solubility profile (Pearson et al 1965, Crenwelge et al 1974). Stabilizing effects of proteins in emulsions were established at all pH values below that of the isoelectric point of the protein and up to pH 6.5. In another study, stable emulsions were prepared at the pH range of 3–10 (Crenwelge et al 1974).

Emulsifying capacity of peanut flour was poorest at pH 4.0, a level near the isoelectric point of the predominant native peanut protein (arachin). At this point, proteins have a net electrical charge of zero and minimum solubility and reactivity (McWatters and Holmes 1979a). Emulsifying capacity of peanut protein was pH- and protein concentration-dependent and was adversely affected by high salt concentration at most pH levels (McWatters and Holmes 1979b, Ramanatham et al 1978). Pearson et al (1965) found that the optimum pH values for emulsification were 9.4 for soy protein, 3.1 for globin, and 8.9 for cottonseed proteins.

The solubility of defatted corn germ protein (CGP) preparations for pH values between 6 and 7 was determined to relate protein solubility to emulsifying capacity and emulsion stability (Lin and Zayas 1987). CGP isolate had good protein solubility at neutral and low pH and the ability to stabilize oil-in-water emulsions (Nielsen et al 1973).

There is very little information related to emulsifying capacity and emulsion stability of defatted CGP. Since 1984, we have conducted studies on the basic factors affecting the functional properties of defatted CGP as an additive in comminuted meat products. Defatted corn germ protein (CGP) contains largely globulins and albumins. Although the individual functionalities of several kinds of corn proteins (Lucisano et al 1984, Peri et al 1983) and emulsifying capacity and emulsion stability of supercritical and hexane-extracted CGP (Lin and Zayas 1987) have been studied, very little work has been done to evaluate the emulsifying properties of hexane-defatted CGP.

The objective of this research was to investigate the emulsifying capacity and emulsion-stabilizing effects of two hexane-defatted CGP preparations in protein-oil-water emulsions in order to obtain information related to emulsifying properties of defatted CGP. Two different defatted CGP were studied, obtained by conventional and modified fat extraction methods.

MATERIALS AND METHODS

Sample Preparation

Two hexane-defatted CGP preparations were used in experiments. One defatted CGP was processed by the conventional method and the other by a modified technique.

Emulsifying Capacity and Emulsion Stability

Despite the complexity of the emulsification mechanism, it is possible to show differences in the emulsifying properties of different corn protein preparations, using simple tests for emulsifying capacity (EC) and emulsion stability (ES). The determination of the emulsifying characteristics of defatted CGP evolved via two main approaches: EC and ES measurements. There is a difference between the EC and ES of emulsions produced with proteins. EC is the ability of the protein solution or suspension to emulsify oil. ES is not a characteristic of maximum oil addition, but rather the ability of the emulsion to remain stable and unchanged. Emulsion with low stability will appear visually as fat separation or creaming, which is caused by flocculation and coalescence. The best method of measuring ES is to register the size distribution of the fat particles as a function of different factors.

Emulsifying capacity was measured by using a model system to test how much oil could be emulsified into an aqueous phase containing the test protein. The method of Satterlee et al (1973)

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was used with modifications. Salt solution (97 ml, 1*M*) was blended in an Oster blender (model 548-41A) with 3 g of CGP at low speed (8,000 rpm) for 1 min. The pH of the resulting solution was adjusted from pH 4.0 to 8.5 with 1*N* HCl or NaOH. Corn oil was added into the blender at a rate of approximately 1 ml/sec. During the emulsification, the direct-current (dc) conductivity of the emulsion was monitored with a dc microampere (μA) meter. The conductivity began with 50 μA, and when the emulsion broke, the dc conductivity rapidly dropped to 20 μA and lower. The end point of adding corn oil was determined at the moment when the current dropped. The amount of corn oil (ml) used was recorded as the EC.

ES was measured by using a model system to test the stability of an emulsion prepared under specific conditions, following the procedures of Inklaar and Fortuin (1969) and Hutton and Campbell (1977). The emulsions were prepared from corn oil (20.00, 26.67, 33.33, and 40.00%), defatted CGP preparations (3.00, 4.33, 5.67, and 7.00%), and water (total 150 g). The defatted CGP and water mixture was incubated at 70° C for 10 min in a water bath. Corn oil at a temperature of 25° C was added to the protein-water solution in an Oster blender (model 548-41A) and emulsified at high speed (20,000 rpm) for 2, 3, 4, and 5 min. The emulsion was held in two 50-g centrifuge tubes and incubated in a water bath at 4, 9.3, 14.7, and 20° C for 30 min. Subsequently, the samples of emulsion were centrifuged at 3,000 rpm for 30 min. The amounts of water separated were presented on a calibrated scale in milliliters. The percentage of water retained in the emulsion was determined according to the equation:

$$ES = \left(\frac{\text{ml of water in emulsion} - \text{ml of water separated}}{\text{ml of water in emulsion}}\right) \times 100$$

Statistical analyses. The ranges and intervals of experimental parameters for response surface methodology (RSM) analysis followed the designs of Box and Behnken (1960) and Box and Wilson (1951). Each experiment was replicated once for each treatment. Data analysis and graphic plotting were done with SAS programs (SAS 1982). Quadratic models were used to create the three-dimensional response surfaces. In response surfaces, independent variables are located along the traditional x- and y-axes, respectively, whereas the response variable is at the z-axis perpendicular to the x-y axes.

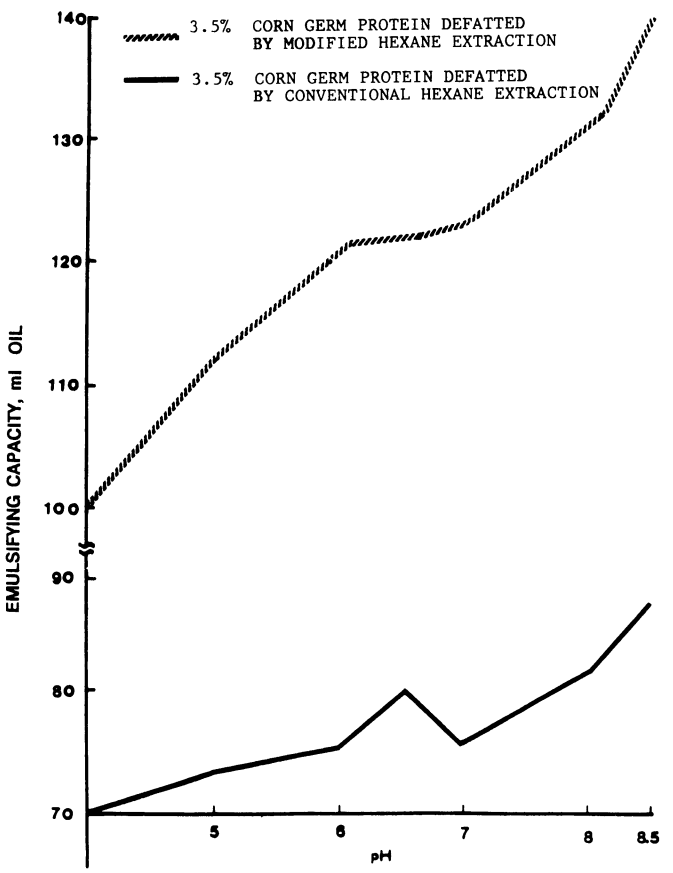

Fig. 1. Emulsifying capacity of corn germ protein defatted by conventional and modified hexane extraction method as a function of pH.

RESULTS AND DISCUSSION

At present, conventional oil extraction by hexane leaves a certain amount of residual lipids in the defatted CGP, which reduces its quality (Phillips and Sternberg 1979). The modified hexane extraction method tested here significantly reduced the amount of residue oil in defatted CGP. As a result of the utilization of modified method, the flavor characteristics and color of defatted CGP were significantly changed. Hexane-defatted CGP had a food-grade quality and considerable potential for use as a supplement in a variety of foods.

Emulsifying Capacity

EC was expressed as the maximum amount of oil that the corn germ protein solution would emulsify without losing its emulsion characteristics. Figure 1 shows the effect of pH on the EC of two hexane-defatted CGP. Both preparations had the lowest levels of EC in their isoelectric region, and EC increased at pH values above this region. As shown in Figure 1, both hexane-defatted CGPs indicated minimum values of EC at pH 4.0–4.5. Defatted CGP reflected the common trend of vegetable proteins to have the lowest values for emulsifying properties in their isoelectric region (Aoki and Nagano 1975, Franzen and Kinsella 1976, Ramanatham et al 1978, Volkert and Klein 1979).

A comparison of Figures 1, 2B, 3B, and 5B calls attention to the fact that there is a relationship between solubility and EC, i.e., the EC of defatted CGPs correlates with their solubility. At a higher pH, protein had higher solubility, which certainly increased the EC of defatted CGP. A significant increase in EC was obtained at a pH range of 7.0–8.5. Hexane defatted CGP obtained by the modified extraction process had a higher EC than that obtained by the conventional process. This was supported by the evidence of higher protein solubility. When protein solubility is optimized, a lower protein concentration is required to obtain oil phase volumes similar to those at neutrality.

Emulsion Stability

An optimal composition of the components is needed to obtain a stable emulsion, with little fat or water separated after holding or centrifugation. Emulsifying capacity and emulsion stability were affected by the emulsifier concentration. Emulsion stability was determined for both defatted CGP samples at concentrations from 3 to 7%. For each sample of defatted CGP, several protein and fat concentrations and emulsifying time combinations resulted in relatively effective emulsification. An increase in protein concentration resulted in increased ES (Figs. 2A, 3A and B, 4A, 5A and B). There was no measurable fat separation in these emulsions. High ES was obtained as the result of defatted CGP specific properties and carbohydrate component of defatted CGP (about 34–38%).

As shown in Figures 2A and 3A, defatted CGP obtained by the modified extraction process had the highest ES, i.e., the least water separation at 7% protein concentration and 40% fat in emulsion ($P < 0.01$). At lower protein concentrations (3–3.5%), emulsions of lower stability were obtained. Centrifugation of these emulsions resulted in higher amounts of separated water. Holding temperature also influenced ES of emulsions (Figs. 3A and 5A).

Lower values of ES were obtained for the conventionally hexane-defatted CGP ($P < 0.01$). Increasing the defatted CGP content increased the ES of the system (Figs. 4A, 5A and B). An important factor influencing EC and ES of conventionally defatted CGP was its higher protein denaturation compared with samples defatted by the modified process. Difference in protein denaturation of samples defatted by modified and conventional processes was established by differential scanning calorimeter

studies (Zayas and Lin, *unpublished data*).

Emulsifying time in the range 2–5 min had no significant effect on ES of emulsions at protein concentrations from 3 to 7% (Fig. 4A). The effect of protein concentration on EC and ES can be explained that as the amount of soluble proteins increased, the thickness of the protein membrane also increased, and consequently ES increased. The similar emulsion stabilities presented in Figs. 2–5 indicate that the protein films were approaching a stable state in the emulsions.

Data related to the effect of protein solubility on EC and ES are

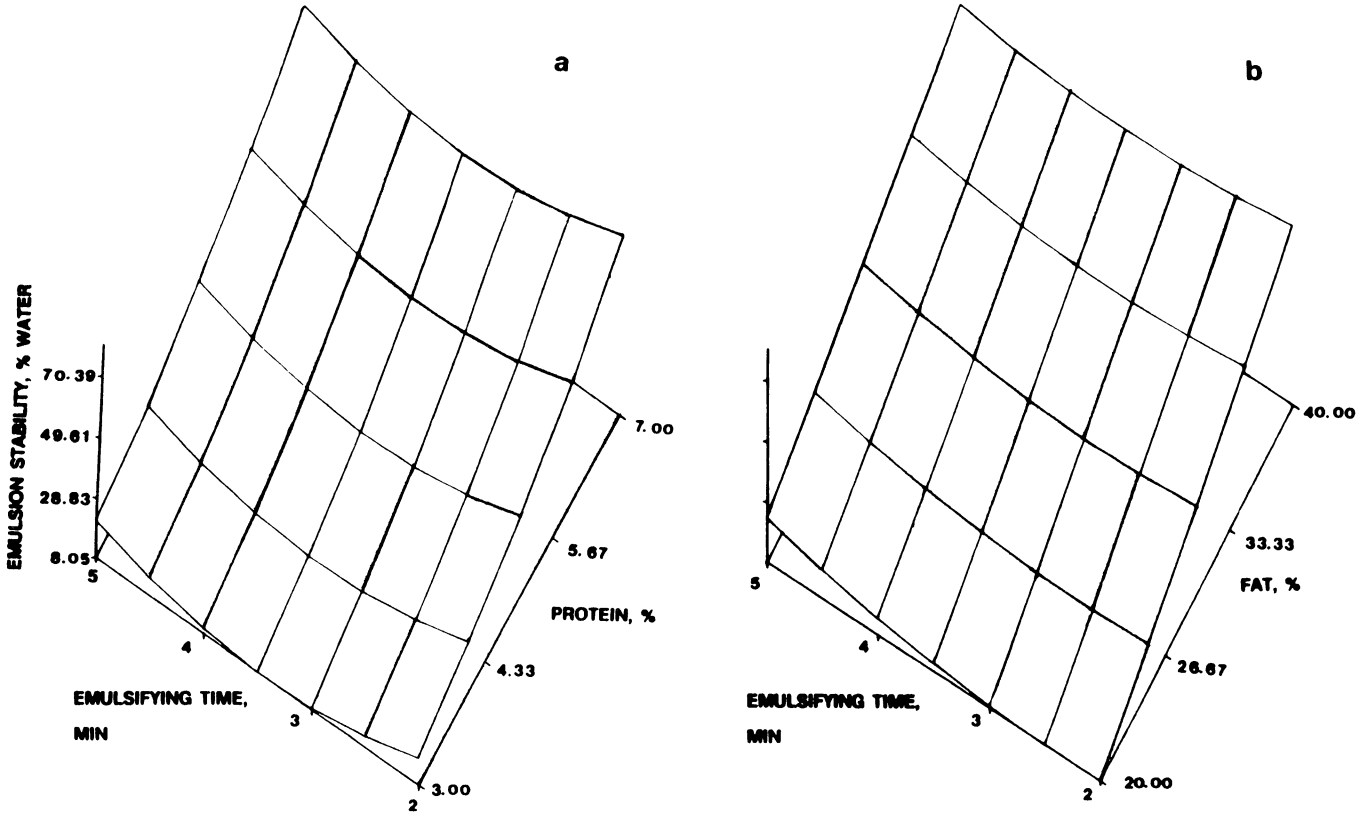

Fig. 2. Emulsion stability of corn germ protein defatted by modified hexane method as a function of: **A**, emulsifying time and protein concentration; and **B**, emulsifying time and fat concentration.

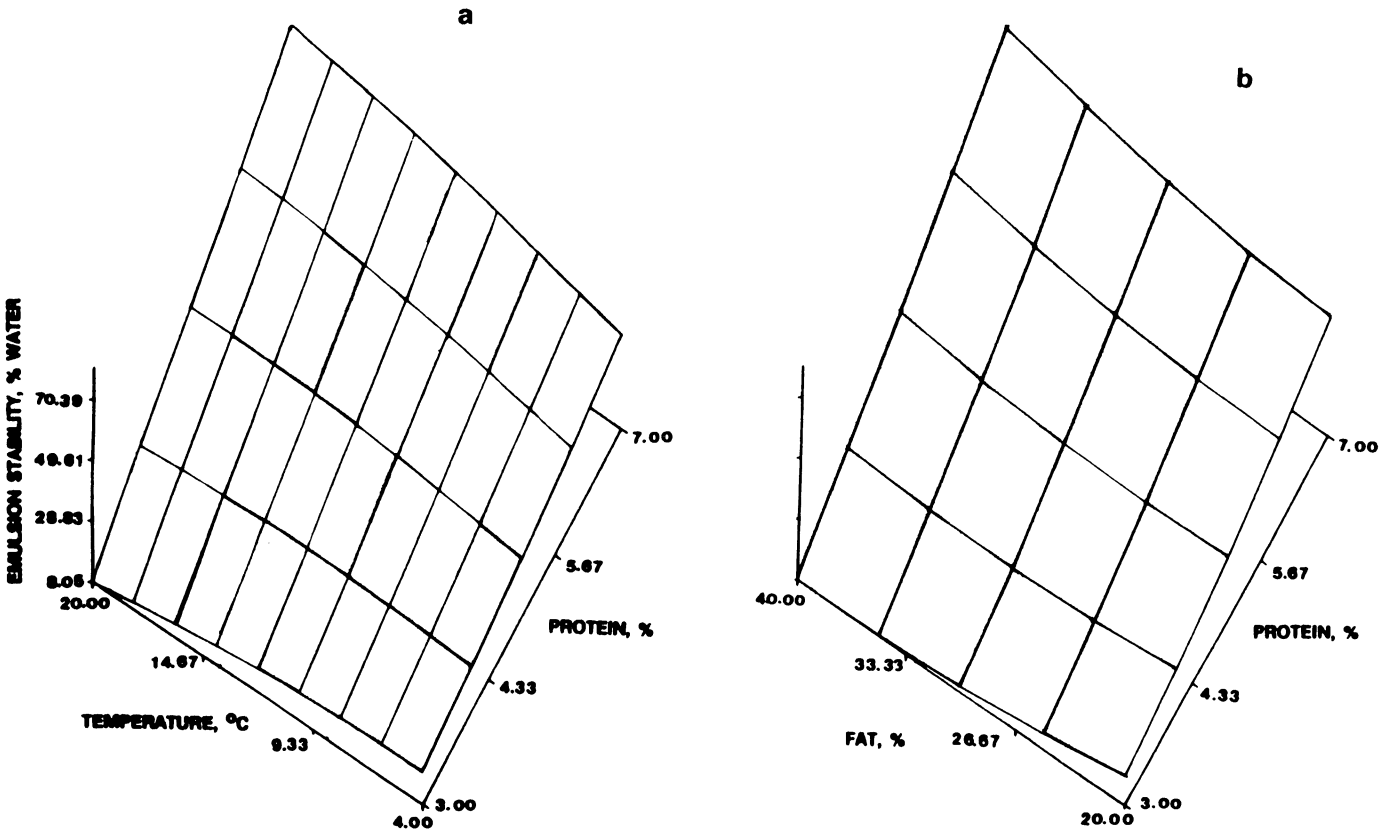

Fig. 3. Emulsion stability of corn germ protein defatted by modified hexane method as a function of: **A**, temperature of incubation and protein concentration; and **B**, fat and protein concentration.

controversial. McWatters and Holmes (1979b) showed that large concentrations of soluble proteins were not necessarily related to maximum emulsifying capacity and emulsion stability. Another study found that emulsifying efficiency decreased rapidly with increases in the concentration of soluble proteins (Ramanatham et al 1978). Undissolved particles of proteins in the aqueous phase of emulsion can also stabilize the system by serving as a physical barrier to coalescence of oil droplets. Protein particles are absorbed at the oil-water interface more quickly than individual protein molecules.

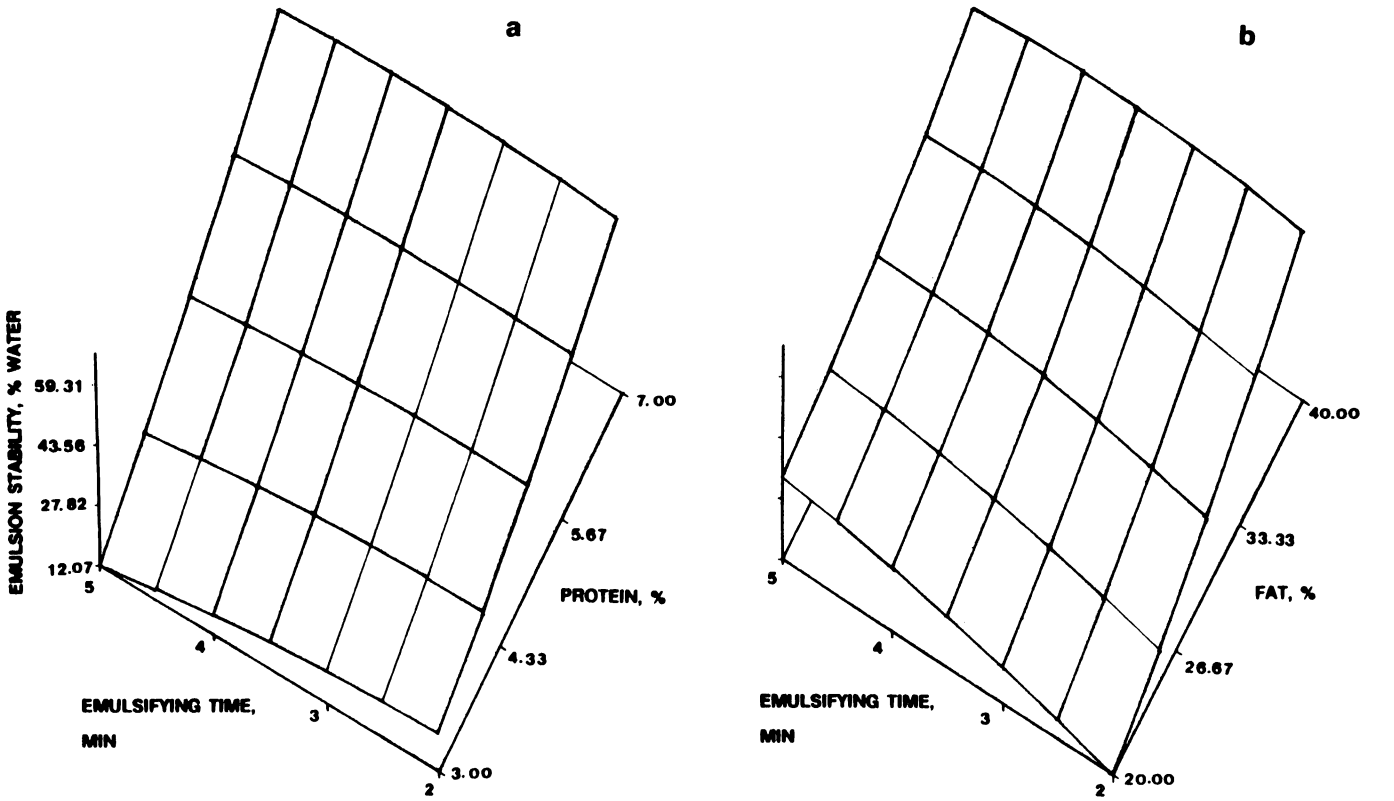

Fig. 4. Emulsion stability of corn germ protein defatted by conventional hexane method as a function of: **A**, emulsifying time and protein concentration; and **B**, emulsifying time and fat concentration.

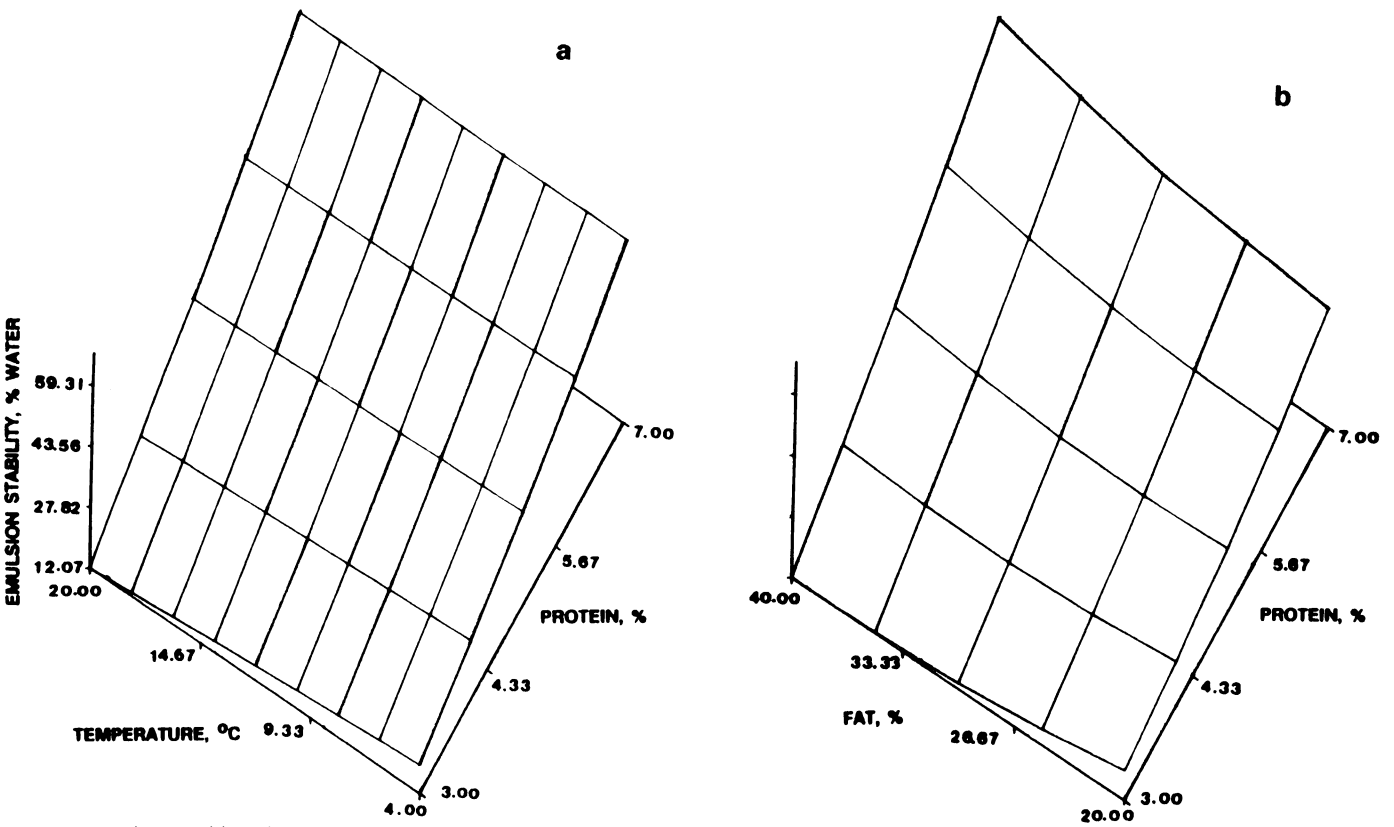

Fig. 5. Emulsion stability of corn germ protein defatted by conventional hexane method as a function of: **A**, temperature of incubation and protein concentration; and **B**, fat and protein concentration.

Constituents of the defatted CGP preparations other than protein aid in formation of emulsions. For example, carbohydrates contributed significantly to the emulsifying stability of defatted CGP. Carbohydrates content in both defatted CGP was in the range of 34–38%. The additional carbohydrates in the defatted CGP samples might have competed successfully for water and, thus, reduced the water available as a dispersion medium during emulsification.

Both fat concentration and emulsifying time contributed to the increased ES of conventional defatted CGP, which was not shown with other factors (Fig. 4B). Incubation temperatures had no significant effect on the ES of defatted CGP (Figs. 3A and 5A). However, at higher incubation temperatures, ES was slightly lower when protein concentration was also low (Figs. 3A and 5A).

Oil Concentration

The emulsified oil concentration response surfaces for defatted CGP obtained by modified and conventional processes are shown in Figures 2B and 4B, respectively. Increasing fat contents up to 40% in the emulsion had a positive effect on ES of both preparations. This positive effect of increased fat concentration was related to increased viscosity of the emulsion. The highest ES was obtained with 70.39% water retained by the emulsion.

Surface responses of ES to increased fat content in the emulsion showed the same trend for defatted CGP processed by modified and conventional methods (Figs. 2B and 4B). Emulsions produced with defatted CGF obtained by the modified extraction procedure consistently had less water and emulsified oil separated after centrifugation than did those containing defatted CGP extracted by the conventional procedure.

Increasing the emulsifying time slightly increased the ES (Figs. 2B and 4B), when the factors of fat content and emulsifying time were studied. However, the predominant factor was protein concentration, which contributed most to the increased ES. The effects of defatted CGP concentration and fat concentration on EC and all two-factor interactions were significant at the level $P < 0.01$ (Figs. 3B and 5B).

The quadratic models for ES of two hexane-defatted CGP were:

$$Y_1 = 69.9259 - 12.2097T - 1.7228I - 1.6461F - 4.336TP + 2.0867T^2 + 0.3015TI - 0.0294I^2 - 0.0148TF + 0.0273F^2 - 1.0917TP + 0.4511IP + 0.2275FP + 0.2578P^2 \quad (P < 0.05,\ R^2 = 0.7901)$$

$$Y_2 = 28.1817 + 8.8094T + 0.2564I - 0.8431F - 12.1323P - 0.8163T^2 + 0.0356TI - 4.9479\text{E} - 4I^2 - 0.2222TF - 0.0293IF + 0.022F^2 + 0.85TP + 0.1045IP + 0.2844FP + 0.5546P^2 \quad (P < 0.001,\ R^2 = 0.9603)$$

where Y_1 and Y_2 were ES of defatted CGPs processed by modified and conventional methods, F = fat content, P = protein content, T = emulsifying time, and I = incubation temperature.

CONCLUSIONS

Functionality of defatted CGP, particularly EC and ES, is important for its use as an additive in food systems. Emulsifying capacity and emulsion stability of defatted CGP can be used to define how defatted CGP can be added to existing foods and how it can replace more expensive proteins traditionally used. Because of that, these properties of defatted CGP are important in evaluating their potential use as food additives. Hexane-defatted CGP obtained by a modified process had higher EC and ES than that obtained by the conventional extraction process. Because of better functional properties in the model system, hexane-defatted CGP obtained by the modified process is recommended for utilization as a food additive.

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