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Protein-Stabilized Emulsions: Effects of Modification on the Emulsifying Activity of Bovine Serum Albumin in a Model System

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A series of laboratory blenders and homogenizers were compared for their capacity to make protein-stabilized emulsions. A Janke-Kunkel blender consistently gave the best emulsions. By use of optimum protein concentrations (0.5%) and temperature (15 °C), the effects of modification on the emulsifying activity (EA) of bovine serum albumin (BSA) over the pH range 2–10 were studied. Reduction of the disulfide bonds reduced the EA, urea (8 M)-eliminated EA, while succinylation significantly enhanced the EA of BSA in the pH range 4–7. These studies indicated the importance of protein structure and charge on emulsifying properties. BSA had superior EA to soy proteins, arachin, β-casein, ovalbumin, and β-lactoglobulin.

Many common foods are broadly classified as emulsions, e.g., milk, ice cream, salad dressing, mayonnaise, and, in some cases, processed meats. In several of these foods proteins serve as the principal emulsifying agents in stabilizing the dispersed oil droplets (Kitchener and Musselwhite, 1968). The criteria frequently used to describe emulsifying properties are emulsifying activity (EA), emulsion capacity (EC), and emulsion stability (ES). The ability of protein to aid in emulsion formation and stabilization is EA, the volume of oil emulsified per unit weight of protein is defined as EC, and the ability of the discrete emulsion droplets to remain dispersed without creaming, flocculating, coalescing, or oiling off is defined as ES. A fixed oil phase volume is necessary for EA and ES, while EC is a function of the amount of oil emulsified.

There is a lot of research concerned with the determination of the emulsifying properties of food proteins. A wide diversity of model systems and varying conditions have been used to determine emulsification properties of numerous proteins, and useful comparisons of methods and results are thus made difficult (Tornberg and Hermansson, 1977; Pearce and Kinsella, 1978; Crenwelge et al., 1974; Acton and Saffle, 1970).

Emulsifying activity reflects the ability of the protein to aid emulsion formation and stabilization of the newly created emulsion (Kitchener and Musselwhite, 1968; Ivey et al., 1970). EA is measured by determining the particle size distribution of the dispersed phase by microscopy, Coulter counting, or spectroturbidity (Walstra et al., 1969). In each procedure an average diameter of the dispersed phase is determined and from these data the interfacial area can be calculated. Results from microscopic techniques take much time and show poor reproducibility, while the Coulter counter method is more reproducible and the lower limit of emulsion particles detected is ~0.7 μm (Walstra et al., 1969).

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The spectroturbidity method is simple, rapid, and theoretically sound (Kerker, 1969) and provides information about the average diameter and particle size distribution. The method is applicable to emulsions with average particle size diameters between 0.2 and 8 μm (Mulder and Walstra, 1974; Walstra et al., 1969). The optical density of diluted emulsions is directly related to the interfacial area (i.e., the surface area of all the droplets) for coarse emulsions (Pearce and Kinsella, 1978).

Several types of blenders and homogenizers and many sizes and shapes of containers have been used in emulsion preparation (Tornberg and Hermansson, 1977; Pearce and Kinsella, 1978; Johnson et al., 1977; Tsai et al., 1970). The valve homogenizer has been used widely (Tornberg and Hermansson, 1977; Berger, 1976; Titus et al., 1968; Klotzek and Leeder, 1966; Sherman, 1965) whereas few emulsion studies have been conducted with sonicators (Tornberg and Hermansson, 1977; Smith and Dairiki, 1975; Higgins and Skanen, 1972). These instruments vary in their ability to form an emusion; i.e., the particle size distribution of the oil droplets vary and frequently factors affecting emulsifying activity are overridden by the characteristics of the equipment used.

For determination of the relative emulsifying properties of proteins and for studying the relationship(s) between protein structure and emulsifying properties, a standardized model system for optimum emulsion formation is needed. Earlier we evaluated a spectroturbidimetric technique for determining emulsifying capacity of proteins (Pearce and Kinsella, 1978). In the present study using bovine serum albumin we compared the relative emulsion-forming characteristics of five laboratory homogenizers and studied the relationship between the structure of BSA and its emulsifying activity.

MATERIALS AND METHODS

Bovine serum albumin (Cohn Fraction V), ovalbumin, β-casein, and β-lactoglobulin were purchased from Sigma Chemical Co. (St. Louis, MO) and used as such. Soya protein isolate was obtained from Ralston Purina Company

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Table I. Effect of Homogenizer on the Particle Size Distribution and Optical Density of Protein-Based Emulsions[a]

homogenizer	homogenization condition[b]	microscopic particle size distribution, %			optical density (550 nm)
		<4 μm	4–10 μm	>10 μm	
Brinkman/Polytron PT-20	25 s	73.0	21.3	5.7	0.229
Waring Blendor (minicontainer)	15 s	75.8	16.8	7.4	0.285
Waring Blendor (semimicrocontainer)	30 s	80.3	14.5	5.3	0.204
hand homogenizer	15 passes	89.2	10.4	0.5	0.676
Janke-Kunkel (Model A10)	30 s	97.0	2.2	0.7	0.817

[a] Emulsions were prepared with 10 mL of peanut oil and 20 mL of 0.50% bovine serum albumin (0.10 M NaCl; pH 7.0; 20 °C). [b] Determined in preliminary studies as the time required to give maximum optical density.

(St. Louis, MO), and arachin was isolated from defatted peanut flour according to the method described by Shetty and Rao (1974). To study relationships between structure and emulsifying properties, we modified batches of BSA in various ways. Bovine serum albumin was denatured with 8 M urea (U.S.P., Mallinckrodt Inc., St. Louis, MO) for 6 h at 37 °C, extensively dialyzed against deionized water, and lyophilized. The disulfide bonds of BSA were reduced by reacting with 0.01 M DL-dithiothreitol (Sigma Chemical Co., St. Louis, MO) for 4 h at 40 °C. The reduced protein was dialyzed against deionized water and lyophilized. Acylated BSA was prepared with acetic anhydride (Mallinckrodt, Inc., Paris, KY) and with succinic anhydride (Eastman Kodak Co., Rochester, NY) as described (Franzen and Kinsella, 1976b). A 10:1 (w/w) ratio of anhydride to protein was utilized to acylate BSA at pH 8–9, 25 °C, for 1 h. The acylated protein was dialyzed against deionized water and lyophilized.

All reagents were of reagent grade and doubly distilled deionized water was used in the preparation of all solutions.

Equipment. Five homogenizer systems were evaluated: a hand-operated homogenizer (Fisher 11-504-200), a Brinkman/Polytron homogenizer with a PT-20 generator, a Waring blender (Model 5011) with a semimicrocontainer (Eberback Model No. 8580) and with a minicontainer (Waring MC-1 run at 20 500 rpm), and a Janke-Kunkel (J-K) Model A10 blender operated at 10 000 rpm. Centrifugation was conducted on a Sorval RC-5 Superspeed Refrigerated centrifuge with a GSA rotor (Du Pont Instruments, Newton, CT).

Emulsification Procedure. Emulsions were prepared with 10 mL of protein solution and 20 mL of peanut oil. For determination of which homogenizer produced the best emulsions, each system was optimized for homogenization time or number of passes by using 0.50% (w/v) bovine serum albumin in 0.10 M sodium chloride (pH 7.0) at 20 °C. Having determined the best instrument for emulsion formation, we studied the effects of different factors on emulsifying capacity. The effect of temperature (2–40 °C) was determined by using the Janke-Kunkel blender (10 000 rpm; 30 s) under identical conditions. The effect of homogenization times at several BSA concentrations (0.10, 0.30, 0.50, 1.25, and 2.00% w/v) was also studied by using the Janke-Kunkel blender (30 s) at 15 °C. The effect of ionic strength was determined by using sodium chloride and sodium sulfate (Mallinckrodt Inc., Paris, KY) with 0.50% (w/v) BSA (pH 7.0) at 15 °C.

The effect of pH on emulsifying properties of modified BSA was determined with acylated protein, 0.50%, in 0.10 N sodium chloride by using the Janke-Kunkel blender for 30 s at 15 °C.

The emulsifying activity of BSA was evaluated by light microscopy and spectroturbidity. The particle size distribution of emulsion droplets was monitored with a microscope at 400× magnification (Carl Ziess, West Germany). The emulsion was diluted 250-fold with 0.10 M sodium chloride containing 0.05% sodium dodecyl sulfate (pH 7.0) to stabilize the dispersed oil droplets. The scale in the eyepiece was calibrated with 0.10-mm scale on a calibration slide and the divisions on the eyepiece were 4 μm apart. The oil droplets were classified into the following size categories: 1–4, 4–10, and >10 μm diameter.

The optical density (OD) of emulsions, diluted 500–3000-fold, were determined with a Bausch & Lomb Spectronic 700 spectrophotometer (Rochester, NY) at 550 nm in a 1-cm glass cuvette. Dilutions were made with 0.05% sodium dodecyl sulfate (Bio-Rad Laboratories, Richmond, CA) in 0.10 M sodium chloride (pH 7.0) to stabilize the dispersed oil droplets (Pearce and Kinsella, 1978).

RESULTS AND DISCUSSION

Homogenization System. Each emulsification system was optimized to provide emulsions with the highest optical density (OD) and the smallest particle size distribution. This was accomplished by varying homogenization time (HT) or number of passes.

The optimum condition for each system resulted in emulsions with different properties (Table I). The homogenizers are listed in the order of decreasing particle size. As predicted from the Mie theory (Kerker, 1969), an increased number of small oil droplets corresponded to an increased value of OD. The lower OD of emulsions prepared with the Waring Blendor (semimicrocontainer) probably reflected the paucity of droplets less than 1 μm.

The Janke-Kunkel homogenizing system produced emulsions with the smallest particle size and greatest OD. The relatively small container and the configuration, size, and speed of rotating blades of this system were responsible for the improved emulsification activity obtained with this equipment. Therefore, this apparatus was used in all subsequent studies.

Effect of Temperature. Because temperature affects protein structure, solubility, diffusion rates (to interface), and viscosity of oil, we studied the effect of temperature on EA. Emulsions were prepared by using 0.50% BSA solution (0.10 M sodium chloride, pH 7.0) using the J-K blender for 30 s of homogenization. Smaller and more numerous emulsion droplets were observed in emulsions prepared between 10 and 20 °C as reflected by the OD (Figure 1).

The rate of diffusion of protein molecules to the newly formed interface and the rate of adsorption and unfolding are factors which affect both the formation of and stabilization of emulsion droplets (Graham and Phillips, 1976; Tornberg, 1979). Temperature may affect both of these phenomena, thereby influencing the observed EA. Thus, at the lower temperatures (below 8 °C) the rate of diffusion and rate, and possibly extent, of denaturation at the oil–water interface were reduced because the kinetic energy, i.e., thermal energy of the dispersion, was reduced (Figure

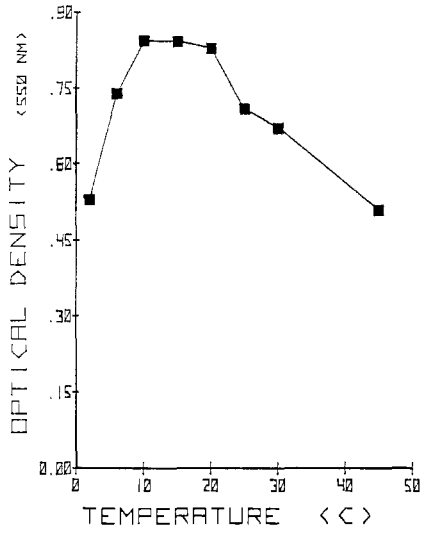

Figure 1. Effect of temperature on the optical density (1:1000 dilution) of emulsions prepared with 0.50% bovine serum albumin.

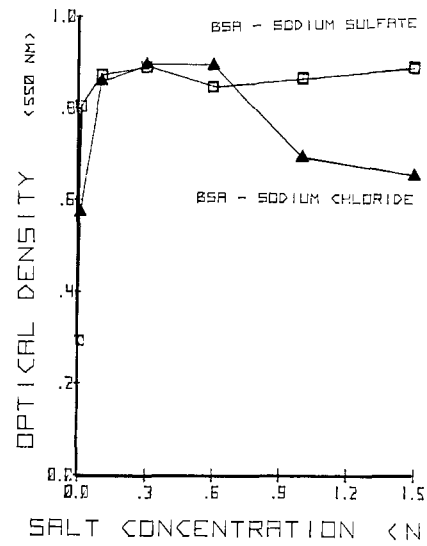

Figure 2. Effect of homogenization time on the optical density (1:1000 dilution) of emulsions prepared by using different concentrations of bovine serium albumin.

1). At higher temperatures (10–25 °C) the increased kinetic energy of the solution caused BSA to adsorb more rapidly and to unfold and form a stable interfacial film which formed a relatively stable emulsion. At the higher temperatures excessive denaturation of the protein with some coagulation may have occurred, thereby reducing the amount of protein available for film formation.

The amount of kinetic energy (heat) in the homogenizing system results from the applied temperature and the mechanical agitation, i.e., shear. Since the amount of mechanical agitation could be varied by changing the homogenizing time (HT), the effect of the amount of kinetic energy imparted to the system on EA was studied. Thus the effects of HT at 2 and 45 °C was evaluated to determine if the extent of mechanical agitation affected EA of emulsions prepared with BSA. The OD of diluted emulsions prepared at 2 °C increased by 18% when the HT was increased from 30 to 45 s (data not shown). This indicated that the greater amount of shear, i.e., the increased amount of kinetic energy, enhanced the EA of BSA at 2 °C by facilitating the adsorption of the protein at the oil–water interface and thereby stabilizing the emulsion. The OD of the diluted emulsion made at 45 °C was increased 15% when the HT was decreased from 30 to 20 s, indicating that the emulsion made by homogenizing for 30 s was overmixed; i.e., too much kinetic energy was supplied to the system. This probably resulted in excessive unfolding of BSA and denaturation of the proteins with concomitant destabilization of the film at the oil–water interface and coalescence of fat droplets. Consequently, the OD of the emulsion was reduced; i.e., the number of oil droplets was reduced. Thus, depending upon the homogenizer used, there is an optimum HT at each temperature.

Higher emulsifying temperatures (40–80 °C) may promote the emulsification of certain food products; however, because the viscosity of both the oil and aqueous phases decreased, the coalescence of the oil droplets may also be promoted (Carpenter and Saffle, 1964; Berger and White, 1971). Smith et al. (1977) and Hutton and Campbell (1977) also reported that the stability of protein-stabilized emulsions was affected by temperature, and the optimum temperature varied with pH, homogenization pressure, and type of protein.

Effect of Protein Concentration. Since the EA depends on the formation of a stable interfacial protein film, increasing the concentration of soluble protein should increase emulsion formation. In our studies the EA progressively increased with protein concentration (Figure 2), especially at homogenization times above 20 s. The homogenization time required to obtain maximum EA (i.e., OD) increased from ~15 to 25 s as the protein concentration was increased from 0.10 to 2.0%, respectively.

The diminishing nonlinear increase in EA with protein concentrations especially above 0.5% reflected the less rapid increase in total interfacial area with availability of protein. MacRitchie (1978) reported that <8 mg of protein was sufficient to provide complete coverage for 1 m^2 of oil–water interface. Our data indicated that an adequate quantity of protein was available to obtain maximum EA in our system at ~1% protein concentration. The additional protein, while not significantly increasing total surface area, made the emulsion more resistant to breakdown as the homogenization time was prolonged. The EA of BSA was slightly reduced with homogenization time, especially at protein concentrations of 0.10–0.50%. This was probably caused by saturation of the oil–water interface with denatured proteins which resulted in coalescence of some droplets (Figure 2). Adsorbed proteins protect and stabilize the interface from transient changes in surface tension caused by shear, vibrations, or temperature fluctuations (MacRitchie, 1978). The shearing occurring during homogenization distorts the dispersed oil globules, causing rupture and coalescence of the oil droplets (Mulder and Walstra, 1974) unless the interfacial film is very strong and viscoelastic or if the continuous phase is very viscous. The shear forces prevailing during homogenization of dilute protein-stabilized emulsions are large compared to the forces stabilizing the interfacial protein film (Mulder and Walstra, 1974). Therefore in the present study some of the proteins adsorbed at the oil–water interface may have been dispersed back into solution during prolonged homogenization. Because protein adsorption is accompanied by denaturation (MacRitchie, 1978), some coagulation of BSA may also have occurred. The net effect would be a reduction in native BSA. Thus, the capacity to form and stabilize an increased interfacial area was reduced as reflected in the lower values of OD with time of homogenization. A slight increase in OD was observed at the protein concentrations above 1.0% because there apparently was a sufficient amount of surface active protein in solution at least during the first 2 min of homogenization.

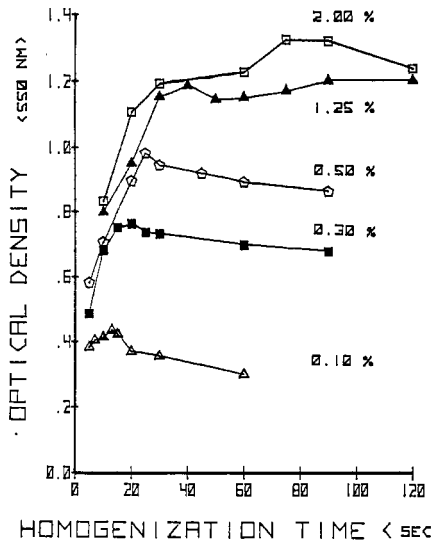

Figure 3. Effect of ionic strength and type of salt on the optical density (1:1000 dilution) of emulsions prepared with 0.50% bovine serum albumin.

These data indicated there was an optimum amount of shear, i.e., kinetic energy, required for the formation of an emulsion. This increased with protein concentration.

Previous research has shown that optimum values for EC and ES were obtained by using intermediate values of blender speed (Carpenter and Saffle, 1964; Ivey et al., 1970; Galluzzo and Regenstein, 1978) or homogenization pressure (Smith et al., 1977).

Effect of Salts. In the formation of an emulsion the protein unfolds to form a cohesive interfacial film (Graham and Phillips, 1976). Thus the molecular flexibility, i.e., facility to undergo conformational changes or unfolding at an interface, affects the EA of proteins. In addition to its inherent properties the conformational stability of proteins is affected by the nature of the environment, e.g., concentration of ions (Kuntz and Kauzman, 1974). Therefore, we studied the effect of two salts, i.e., sodium chloride and sodium sulfate, on the emulsification properties of BSA. These salts were selected because they vary in their affects on the conformational stability of protein (Van Hippel and Wang, 1965).

The ionic strength of the aqueous medium significantly affected emulsification properties of BSA (Figure 3). Solutions of BSA containing <0.02 N sodium chloride or sodium sulfate failed to give maximum optimum EA. Conceivably, at low ionic strengths, the increased charge repulsion at the oil–water interface reduced the amount of BSA adsorbed at the interface. Previous studies of the interfacial behavior of BSA and other proteins indicated that increased charge repulsion reduced the rate of adsorption, the amount of protein adsorbed, and the rheological properties, i.e., strength, of the adsorbed film (MacRitchie, 1978; Ivey et al., 1970; Benjamin et al., 1975; Phillips, 1977). Consequently, the limited availability of interfacial protein at <0.02 N salt limited the formation of emulsion droplets.

The EA of BSA was increased at intermediate ionic strengths of sodium chloride and sodium sulfate (0.1–0.6 N) (Figure 3). The surface charges of BSA at the oil–water interface probably were neutralized at these concentrations of salts which reduced electrostatic repulsion and facilitated an increased rate of protein adsorption and greater protein–protein interactions (MacRitchie, 1978; Phillips, 1977; Kinsella, 1976). Thus the interfacial concentration and the rheological properties of the film were probably maximum under these conditions.

At high ionic strengths (>0.75 N) the EA of BSA was decreased in the presence of sodium chloride and increased in the case of sodium sulfate (Figure 3). Above 0.7 N, sodium chloride (a water structure breaking salt) probably increased the solubility of BSA. The rate of protein (BSA) transfer to the oil–water interface was reduced and the EA of BSA was thereby reduced at a higher concentration of sodium chloride. In contrast, sodium sulfate (a water structure promoting salt) probably maintained the rate of transfer of BSA from the bulk phase at the higher concentration. The increased water structure stabilized the conformation of BSA and enhanced "salting out" or the transfer of BSA from the aqueous phase to the interface. This provided a continuous supply of native molecules of BSA for adsorption at the oil–water interface at the higher concentrations of sodium sulfate, resulting in higher OD. The data indicated that the concentration of salt and the water-structuring properties of salts, by influencing protein solubility and conformation, affect the emulsifying properties of food proteins.

Similar effects have been observed with other emulsion systems. Thus the "salting in" effect of 1.0 M sodium chloride significantly improved the EC of soy flour and peanut flour between pH 4 and pH 6 (McWatters and Holmes, 1979a,b). Muscle proteins, myosin and actomyosin, are insoluble at low ionic strength and soluble at intermediate levels (Galluzzo and Regenstein, 1978). The EC of muscle proteins is enhanced by the addition of salt which increases the soluble protein concentration (Hegarty et al., 1963; Swift and Sulzbacher, 1963; Galluzzo and Regenstein, 1978). Emulsion properties of ice cream mixes are influenced by ionic strength which affect the aggregation properties of the casein (Berger, 1976; Berger and White, 1971). Since some processed foods may contain up to 0.6 M sodium chloride (Galluzzo and Regenstein, 1978), the salt composition of the aqueous phase can affect the quality and stability of emulsions in food products.

Effect of Chemical Modification of Bovine Serum Albumin. The emulsifying properties of proteins are influenced by the composition and structural characteristics of the protein (MacRitchie, 1978; Kinsella, 1976). The effect of different structural modifications of BSA on its EA were evaluated by using conditions determined to be optimum and reproducible for BSA (temperature, 15 °C; ionic strength, 0.5 N NaCl; homogenization time, 20 s in a J-K blender). The EA of modified BSA was determined over a range of pH values because this indicated the effect of net charge on EA (Figure 4).

The EA of BSA was markedly improved above pH 4. The good EA of BSA probably resulted from the unique balance of its chemical and structural properties. BSA is relatively hydrophobic (Kato and Nakai, 1981). Hydrophobic interactons are important in the formation of protein films at the oil–water interface (Graham and Phillips, 1980s–c; MacRitchie, 1978). In addition, the conformation of BSA is altered at the oil–water interface (Graham and Phillips, 1980c), indicating that the conformation of BSA is not rigid because some unfolding of the tertiary structure of protein at the interface improves the characteristics of the interfacial film (Figure 4). The molecular flexibility of BSA enhances its surface active properties (MacRitchie, 1978), and this was reflected in the high OD values obtained (Figure 4).

At pH 4 BSA undergoes an acid-induced conformational transition in tertiary structure; i.e., there is molecular expansion, presumably caused by electrostatic repulsion which results in disruption of hydrophobic bonds (Leonard

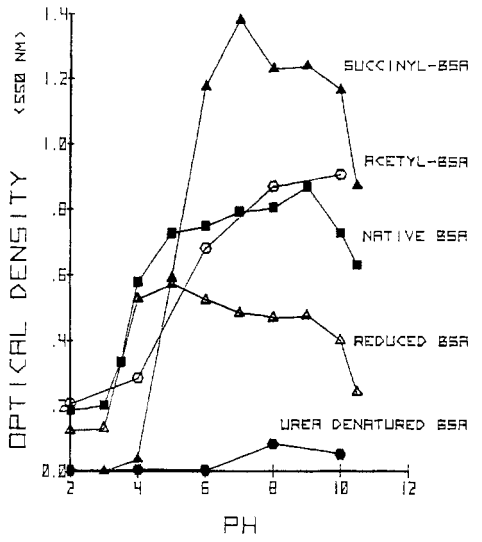

Figure 4. Effect of pH on the optical density (1:1000 dilution) of emulsions prepared with derivatives of bovine serum albumin (BSA). The emulsifying properties of reduced, urea-denatured, acetylated, and succinylated derivatives of BSA were evaluated.

and Foster, 1961; Aoki and Foster, 1957). Our data show a rapid drop in EA below pH 4, indicating that small changes in protein conformation significantly affect EA. This effect is accentuated in the succinylated BSA. These data indicate that electrostatic and hydrophobic interactions are important in EA.

At pH 9 BSA undergoes an alkaline expansion (Leonard and Foster, 1961) which may involve some disulfide cleavage and disruption of hydrophobic attractions (Aoki et al., 1974). The EA of BSA abruptly decreased at pH 9.0, reflecting the altered tertiary structure as disulfide bonds were broken. These data reflect the importance of a certain degree of structural stability (tertiary structure) for optimum emulsion formation.

Because the native structure and conformational flexibility of BSA imparts surface activity, we examined the effects of modification of its tertiary structure on its emulsifying activity. BSA has 17 disulfide bonds which impart a certain degree of structural stability (Brown, 1975). Reduction of these bonds permitted the BSA to unfold, giving a more expanded conformation. This resulted in a decrease in EA (in the pH range pH 4–pH 10) compared to that of the native BSA (Figure 4). This suggested that the native BSA with more tertiary structure formed a stronger, more cohesive interfacial film than did the less folded, reduced BSA. Furthermore, the EA of reduced BSA was more sensitive to pH changes compared to that of native BSA, indicating greater response to electrostatic repulsions, particularly at the higher pH values. Complete disruption of the tertiary and secondary structure of BSA by urea eliminated its EA except for slight activity at alkaline pH (Figure 4).

These data reflect the importance of the native tertiary and secondary structure of proteins for the formation of a stable interfacial film which is required for emulsion formation.

Modification of lysine residues of BSA using succinic anhydride or acetic anhydride to alter the electrostatic interactions markedly affected the EA. Above pH 5 the EA of succinylated BSA (>90 lysine residues succinylated) was markedly improved compared to that of native BSA. This may reflect the increased solubility and somewhat looser structure of the modified protein (Kinsella and Shetty, 1979) which facilitated its diffusion to interface and rearrangement within the interfacial film. Succinylation of several proteins improved their emulsifying properties (Kinsella and Shetty, 1979; Franzen and Kinsella, 1976a,b; Childs and Park, 1976). The decreased EA at higher pH values was probably caused by the marked increase in the electrostatic repulsion between the succinylated polypeptides.

Acetylation, which resulted in elimination of the cationic ϵ-NH_2 groups with little effect on protein solubility, slightly reduced EA between pH 4 and pH 7.

Similar emulsification results were observed with acetylated cottonseed, leaf, and soybean proteins; i.e., there was no change in emulsifying properties after these proteins were acetylated (Childs and Park, 1976; Franzen and Kinsella, 1976a,b).

These data indicate that the EA of food proteins are affected by solubility, secondary and tertiary conformation, and ionic and hydrophobic forces. The relative importance of each of these and the effect of the nature of the continuous phase on EA need to be systematically evaluated by using some of the approaches employed by Graham and Phillips (1980a–c).

Using the model system described in this paper, we also evaluated the EA of several other food proteins. Under optimum conditions and at pH 6.5, relative EA values of 1.0, 0.5, 0.32, 0.30, 0.24, 0.11, and 0.1 were obtained for BSA, 11S fraction of soy protein, ovalbumin, β-casein, β-lactoglobulin, arachin, and soy isolate, respectively. In these studies BSA was consistently the best protein for the formation of emulsions.

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Characterization of Poultry Byproduct Meal Flavor Volatiles

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The vacuum distillate of poultry byproduct meal (PBPM), a pet food ingredient, has been analyzed by WCOT glass capillary gas–liquid chromatography–mass spectrometry. A total of 41 components were identified in this volatile oil. Major components were hexanal, 3-octen-2-one, 1-pentanol, pentanal, heptanal, octanal, 1-heptanol, 1-octanol, and 1-octen-3-ol. Other important odor compounds were 2-(*n*-pentyl)furan, 3,5-octadien-2-one, 3,5-undecadien-2-one, and 2-octenal. These compounds contribute to the notes characteristic of PBPM which may play a role in the acceptance of PBPM-containing pet foods by pet and pet owner.

Poultry byproduct meal (PBPM) is a relatively inexpensive protein source that was first reportedly used in pet foods by Morris (1946) and in the diets of other fur-bearing animals by Bassett and Wilke (1948). It is also used in poultry diets as described by Potter and Fuller (1967). PBPM is prepared out of head, legs, viscera, feathers, lungs, etc. which are waste materials of poultry processing plants. The processing and utilization of PBPM have been the subject of reviews by Rao and Mahadevan (1976) and McNaughton et al. (1977a,b).

The composition of PBPM has been reviewed by McNaughton et al. (1977a) and Doty (1969). These authors found PBPM to contain 54–63% protein, 14–25% crude fat, and 6–11% moisture. Amino acid composition studies indicated that glutamic acid and aspartic acid were the most abundant amino acids. Most of the sulfur amino acids were in the form of methionine and cystine. The flavor chemistry of PBPM or other meat by products which are utilized in pet foods has not been reported in the literature. This is in contrast to the flavor chemistry of prime meats which are used in human consumption and to a limited extent in pet foods. For example, studies of the flavor chemistry of prime meats have been reviewed by Dwivedi (1975) and more recently by Wasserman (1979) and Shibamoto (1980). The flavor of specific meats such as chicken have been reviewed by Wilson and Katz (1972).

It is the objective of this study to identify key flavor components in PBPM. A discussion of how these PBPM components may be formed and how they differ from volatiles of prime meats which have been reported on in the literature will also be presented.

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EXPERIMENTAL SECTION

Materials. PBPM was obtained from a major supplier (Rockingham Poultry Market Cooperative Inc., Broadway, VA). The meal was refrigerated within 1 week after purchase. The PBPM was used "as is" during the isolation procedures.

Authentic chemical reference compounds were obtained from reliable commercial sources (e.g., Aldrich Chemical Co. and Alpha Chemical Co.).

Isolation of the Volatile Flavor: Vacuum Degassing. The volatile flavor components were isolated from a total of 28 lb of Rockingham Poultry By Product Meal by using a vacuum degassing technique.

Each isolation involved degassing 1600 g of poultry byproduct meal at room temperature with a pressure of 0.02 mmHg and collecting the volatiles in a series of coiled traps as those described by Chang et al. (1977) which were immersed in dry ice–2-propanol. At the end of 6 h the volatiles were taken up in diethyl ether. The ether was dried over anhydrous sodium sulfate, concentrated to 0.5 mL by using a Kuderna-Danish concentrator, and concentrated to a final volume of 0.3 mL under a nitrogen stream.

Capillary GLC–Mass Spectral (GLC–MS) Analysis. The two GLC columns used were 50 m × 0.50 mm i.d. Pyrex WCOT glass capillary coated with SE-30 or OV-225. A number of different GLC-MS runs were made with the two columns by using a Hewlett-Packard 5840A gas chromatograph.

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