Singhal, J. P.; Khan, S.; Bansal, O. P. *J. Agric. Food Chem.* **1977**, *25*, 377.
Singhal, J. P.; Khan, S.; Bansal, O. P. *Analyst (London)* **1978**, *103*, 872.
Thean, J. E.; Fong, W. G.; Lorenz, D. R.; Stephens, T. L. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 15.
Townshend, J. L.; Potter, J. W. *Can. J. Plant Sci.* **1979**, *59*, 519.
Truelove, B.; Rodriguez-Kabana, R.; King, P. S. *J. Nematol.* **1977**, *9*, 326.

Received for review March 3, 1982. Revised manuscript received August 9, 1982. Accepted September 9, 1982. This paper was presented at the 182nd National Meeting of the American Chemical Society, Division of Pesticide Chemistry, New York, NY, Aug 1981.

A Simple Turbidimetric Method for Determining the Fat Binding Capacity of Proteins

Leandros P. Voutsinas and Shuryo Nakai*

A simple turbidimetric method was developed for determining the fat binding capacity (FBC) of various proteins. The turbidity was dependent on wavelength, blending time, and volume of oil. The FBC was positively affected by surface hydrophobicity (S_0) and negatively affected by the interaction of S_0 with solubility (s). A highly significant correlation ($R^2 = 0.802$, $P < 0.01$) was found between S_0, $S_0 \times s$, and FBC of 11 food proteins tested. Advantages of the method developed include a small amount of sample required for FBC determination and the fact that the measured values would reflect the true fat binding capacity of proteins by minimizing the fat-entrapping effects.

The ability of proteins to bind fat is an important functional property for such applications as meat replacers and extenders, principally because it enhances flavor retention and reputedly improves mouth feel (Kinsella, 1976). The key role of fat in food flavoring has been demonstrated by Kinsella (1975), and its capacity to improve flavor carry-over in simulated foods during processing is apparent. Soy proteins have been added to ground meats to promote fat absorption or fat binding and thus decrease cooking losses and maintain dimensional stability in the cooked product (Wolf and Cowan, 1975). Fat separation is a well-known major problem in processed meat-in-sauce-or-gravy type products. This problem can be prevented by incorporating into these products (canned or frozen meat/sauce products) a combination of soy protein ingredients (i.e., an extruded soy protein concentrate, a soy protein isolate, and lecithin) designed to emulsify, bind, and stabilize fats (Morris, 1980). On the other hand, in some foods such as pancakes and doughnuts, the addition of soy flour helps to prevent excessive absorption during frying (Johnson, 1970).

Fat absorption of proteins is usually measured by adding excess liquid fat (oil) to a protein powder, thoroughly mixing and holding, centrifuging, and determining the amount of bound or absorbed oil, which is total minus free (Lin et al., 1974; Wang and Kinsella, 1976). The amount of oil and protein sample, kind of oil, holding and centrifuging conditions, and units of expression have varied slightly from one investigator to another (Hutton and Campbell, 1981).

The mechanism of fat absorption is not clear. However, Wang and Kinsella (1976) have attributed fat absorption, as assessed by the above method, mostly to physical entrapment of the oil; in support of this a correlation coefficient of 0.95 was found between bulk density and fat absorption by alfalfa leaf proteins. Chemical modification of protein, which increases bulk density, concomitantly enhances fat absorption (Franzen, 1975).

The objective of this study was to develop a simple method for determining the ability of proteins to bind fat. An effort was made to avoid the entrapment of oil by proteins in order to measure the amount of oil truly bound to the proteins. The development of the method and a comparison of the fat binding capacities of several food proteins are presented.

MATERIALS AND METHODS

Materials. Bovine serum albumin (No. A-4503), β-lactoglobulin (No. L-6879 from milk), and ovalbumin (No. A-5503) were all purchased from Sigma Chemical Co., St. Louis, MO. Soy protein isolate was obtained from General Mills, Inc., Minneapolis, MN. Promine D was purchased from Central Soya Co., Chicago, IL. Pea protein isolate (M 412-046), Century cultivar field pea, was received from POS Pilot Plant Corp., University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Rapeseed protein isolate and sunflower protein isolate were prepared by the method of Nakai et al. (1980). Gelatin, Bloom 300, was purchased from United States Biochemical Corp., Cleveland, OH. Whey protein concentrate (75%) was obtained from Sodispro Technology, St. Hyacinthe, Quebec, Canada. Whole casein was prepared by the method of Voutsinas et al. (1982). Corn oil was from Fisher Scientific Co., Fair Lawn, NJ. Urea, ACS reagent, 99+%, was obtained from Aldrich Chemical Co. Inc., Milwaukee, WI. Metaphosphoric acid was from J. T. Baker Chemical Co., Phillipsburg, NJ.

Fat Binding Capacity Determination. To 40 mg of freeze-dried protein sample in a 15-mL glass centrifuge tube 1.5 mL of corn oil was added. The contents were stirred and sonicated, with a Braun-Sonic 1510 sonicator (Braun Instruments, San Francisco, CA) fitted with a needle probe, at 100 W for 1 min to disperse the protein sample. After being held at room temperature for 30 min the tube was centrifuged at 3020g for 20 min. The free oil was pipetted off and 2 mL of distilled water was added. Oil adhered to the sides of the tube was removed by

Department of Food Science, The University of British Columbia, Vancouver, British Columbia, Canada V6T 2A2.

0021-8561/83/1431-0058$01.50/0

scraping the sides with a glass rod. Then, in order to remove any oil that might have been entrapped in the form of film beneath the protein precipitate, the precipitate was gently scraped from the bottom of the tube and any oil found was taken to the top (i.e., surface of water) with the glass rod. Subsequently, 1 mL of 0.1 N metaphosphoric acid (pH 2.1) was added and the tube was centrifuged at 4340g for 15 min. The supernatant was pipetted off. The precipitate was, then, carefully washed with distilled water (3–4 mL) without dispersing it. The supernatant was pipetted off. Finally, the tube walls were cleaned with a disposable (paper) wiper (to remove any trace of oil, if any existed). After 0.3 mL of distilled water was added, the content of tube was mixed well with the glass rod. A disgestion medium of 20 mL of 7 M urea in 50% H_2SO_4 was measured into a graduated cylinder. An aliquot of about 2 mL of this digestion medium was added into the tube, and the contents were mixed well with a glass rod and then transferred into a Omni-mixer homogenizing chamber. The centrifuge tube was washed twice with about 2 mL of digestion medium. These washings and the remainder of the digestion medium in the graduated cylinder were poured into the homogenizer chamber. The mixture was homogenized for 30 s at speed setting 1 and then poured into a 50-mL beaker.

The sample was held for 30 min at room temperature and then the absorbance was taken at 600 nm in a Spectronic 20 (Bausch & Lomb, Rochester, NY), spectrophotometer with a round cuvette against the digestion medium. The absorbance was stable for at least 1 h. The volume (milliliters) of oil bound was determined from the standard curve. The protein content of the combined supernatants (no. 2 and 3) was, subsequently, determined by the phenol–biuret method (Brewer et al., 1974) to calculate the amount of protein lost in these supernatants during the handling of the precipitate. The amount of lost protein was converted to the amount of the original sample, since the protein content of the sample was known, and this value was subtracted from the 40 mg of the starting sample. This calculation gave the amount of oil in milliliters bound by the corrected amount of protein sample. The fat binding capacity of the sample (expressed as percent) was then calculated as the volume of oil in milliliters bound by 100 g of protein sample.

The standard curve was constructed as follows: measured amounts of corn oil ranging from 0 to 100 mg were added to a series of 40-mg samples of soy protein in 30-mL beakers. While the mixture was being mixed, with a glass rod, 0.3 mL of distilled water was added (to facilitate mixing) followed by 20 mL of digestion medium (7 M urea in 50% H_2SO_4) and further mixing. The mixture was transferred into a Omni-mixer chamber and homogenized for 30 s at speed setting 1 and then poured into a 50-mL beaker. The sample was held for 30 min at room temperature and the absorbance was then taken at 600 nm in a Spectronic 20 with a round cuvette against the digestion medium.

Protein (Surface) Hydrophobicity Determination. Protein surface hydrophobicity was fluorometrically determined according to the method of Kato and Nakai (1980) after slight modification. Each protein sample (2 mL) was serially diluted with 0.01 M phosphate buffer, pH 7.4, to obtain protein concentrations ranging from 0.00156% to 0.05%. Two sets of protein samples were prepared (i.e., two tubes for each protein concentration). Ten microliters of *cis*-parinaric acid solution was added only to one set of tubes. The parinaric acid–protein conjugate was then excited at 325 nm and the relative fluorescence intensity was measured at 420 nm in an Aminco-Bowman spectrofluorometer, with a slit width of 0.5 mm. The method was standardized by adjusting the relative fluorescence intensity reading of the fluorometer to 7.4/10 full scale when 10 μL of *cis*-parinaric acid solution was added to 2 mL of decane. Then, the fluorescence readings of the protein samples were taken. The net fluorescence intensity at each protein concentration was determined by subtracting the fluorescence intensity of each sample without *cis*-parinaric acid from the fluorescence intensity of the corresponding sample containing *cis*-parinaric acid. The initial slope (S_0) of the fluorescence intensity vs. protein concentration plot was used as an index of the protein surface hydrophobicity. The initial slope was determined by linear regression analysis using a Monroe (Orange, NJ) 1880 programmable calculator.

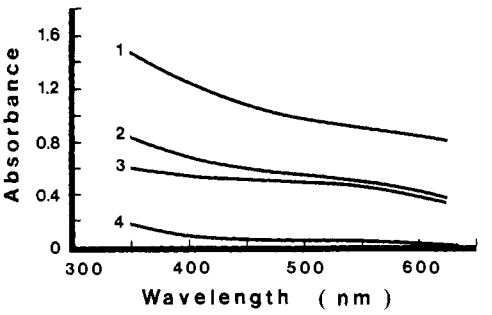

Figure 1. Effect of wavelength on absorbance. (Plot 1) Forty milligrams of soy protein isolate and 50 μL of corn oil were mixed in 20 mL of digestion medium and then homogenized for 30 s. The absorbance was measured after 30 min in a Beckman DB spectrophotometer. (Plot 2) The same as plot 1 except that the absorbance was measured in a Spectronic 20 spectrophotometer. (Plot 3) The same as plot 2 but without protein. (Plot 4) The same as plot 3 but without oil.

Solubility Index Determination. Protein samples (1%, w/v, in 0.01 M phosphate buffer, pH 7.4) were dispersed by stirring with a magnetic stirrer for 5 min and then blended in a Sorval Omni-mixer at speed setting 5 for 1 min. The pH of each dispersion was adjusted to 7.4 by adding 1 N NaOH. For 100%-soluble proteins the blending step was eliminated. A portion of each protein suspension was then centrifuged at 27000g for 30 min. Aliquots of the suspension and the supernatant after centrifugation were diluted and the protein contents were determined by the phenol–biuret method (Brewer et al., 1974). The percent solubility index (s) was taken as the ratio of the protein content of the supernatant to that of the suspension.

Statistical Analysis. Simple and multiple linear regression analyses were performed by using a Monroe 1880 programmable calculator. Backward stepwise multiple regression analsis and surface visualization plotting were done at the University of British Columbia with an Amdahl 470 V/8 computer.

Independent variables used in the backward stepwise regression analysis included surface hydrophobicity (S_0), solubility index (s), interaction of S_0 and s, and quadratic powers of S_0 and s. The model for the prediction of the FBC of food proteins was selected on the basis of the statistical significance of F probabilities of the partial regression coefficients.

RESULTS AND DISCUSSION

Wavelength Dependence of Turbidity. Figure 1 shows the effect of wavelength on absorbance of oil–protein systems. The absorbance values read from the Beckman DB spectrophotometer were higher than those measured by using the Spectronic 20 spectrophotometer. This dif-

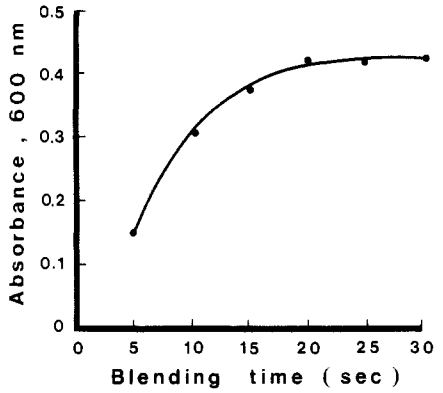

Figure 2. Effect of blending time on absorbance at 600 nm.

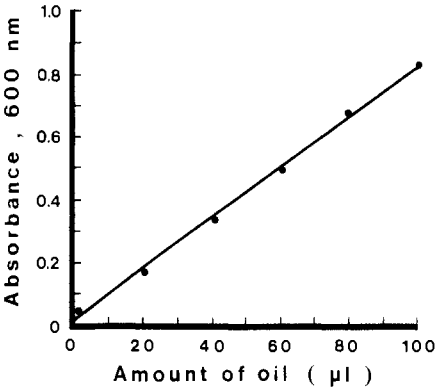

Figure 3. Standard curve for fat binding capacity determination.

ference is probably due to the different cuvette-to-photodetector distance and thus to the different angle of acceptance of the light by the photodetector as suggested in a study of turbidimetry by Pearse and Kinsella (1978). It is also evident from Figure 1 that as the wavelength was increased the absorbance by all samples decreased. In the present study a wavelength of 600 nm was used because of the negligible absorbance by the protein. Moreover, the Spectronic 20 spectrophotometer with a round cuvette was chosen over the Beckman DB spectrophotometer since the latter gave nonreproducible absorbance values. A similar phenomenon was observed by Nakai and Le (1970), who attributed the ability of round cells to give reproducible readings to their focusing effect.

Turbidity Dependence on Blending Time. The effect of blending time during fat binding capacity determination on the absorbance at 600 nm is shown in Figure 2. A gradual rise in absorbance and then attainment of a steady state with increasing blending time were observed. In this study a blending time of 30 s was chosen.

Standard Curve for FBC Determination. The standard curve obtained is shown in Figure 3. The regression equation was $y = 0.008x + 0.012$, where y is the absorbance at 600 nm and x is the amount of bound oil in microliters. The correlation coefficient (r) was 0.9996 and the standard error of estimate (S_{yx}) 0.008.

Comments on the Method for FBC Determination. A flow diagram of the developed method is shown in Figure 4. The use of 0.1 N metaphosphoric acid was necessary for preventing solubilization, thereby losing soluble proteins (e.g., β-lactoglobulin, BSA, whey, and casein) during their resuspension in water (after the first centrifugation). Initially, other protein precipitants (e.g., ethanolic solutions, mercuric chloride, acetate buffer, pH 4.6, and 8% silicotungstic acid in 1.2 M perchloric acid) were added after protein resuspension to precipitate the solubilized protein. These precipitants were found, however, to be

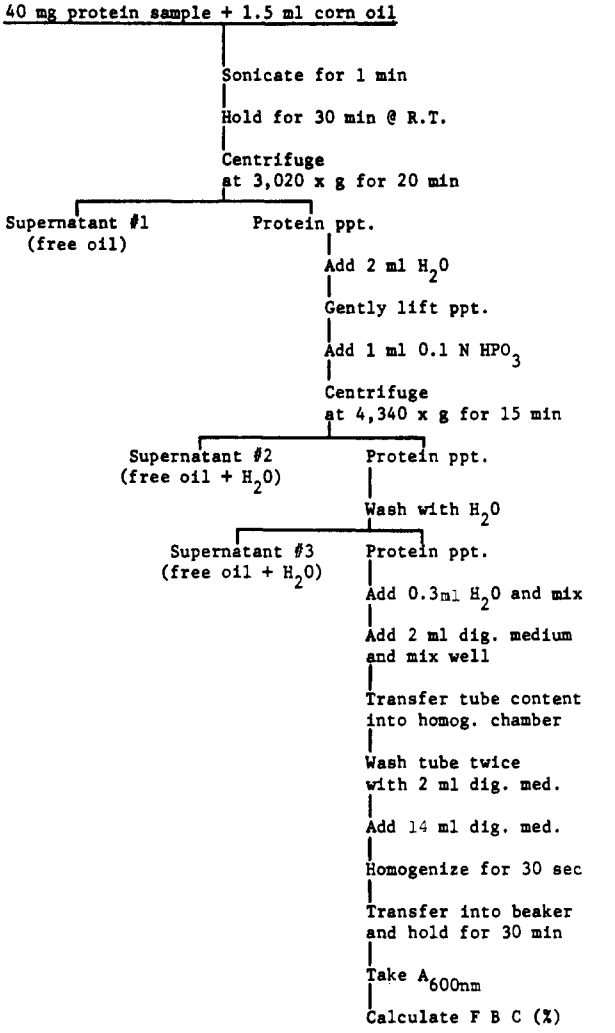

Figure 4. Flow diagram for the determination of FBC of proteins.

unsuitable since the protein precipitate obtained was very firm and not dispersible by the subsequent homogenization step or because of the nonreproducible absorbance reading obtained (silicotungstic acid). Trichloroacetic acid in high concentrations (10–12%) was found effective in precipitating the soluble proteins tested, and their precipitates were easily dispersed in the digestion medium. However, when 10% Cl_3AcOH was used for FBC determination of relatively insoluble proteins (e.g., soy protein), the protein precipitate obtained was very firm and difficult to disperse. Another problem associated with the use of Cl_3AcOH as a protein precipitant is that Cl_3AcOH possesses a hydrophobic group, and therefore, it may react with hydrophobic sites of the protein, thus releasing some of the bound oil. The use of protein precipitants may probably be criticized because many of them (such as Cl_3AcOH, picric acid, and salicylsulfonic acid) cause protein denaturation (Perlmann and Herrmann 1938), which may influence the fat binding by proteins. Thus, metaphosphoric acid, a known strong protein precipitant, was used. It was found very efficient in precipitating soluble proteins (causing instantaneous formation of a precipitate), and moreover, all protein precipitates obtained were easily dissolved by the digestion medium. However, the main advantage of metaphosphoric acid as a protein precipitant is the fact that metaphosphoric acid has been repeatedly shown not to cause protein denaturation (Briggs, 1940; Perlman, 1938; Perlmann and Herrmann, 1938). Briggs (1940) concluded that the metaphosphate–protein reaction could be regarded as a complex in which the negative multivalent (polymerized)

Table I. Relationship between Protein Hydrophobicity, Solubility Index, and Fat Binding Capacity of Various Proteins[a]

protein sample	hydrophobicity (S_0)	solubility index (s), %	FBC,[b] %[c] I	FBC,[b] %[c] II
ovalbumin	6.0	100.0	37.7	560.0
casein	28.0	100.0	10.1	95.0
soy protein isolate	95.0	26.4	105.9	161.0
Promine D	39.0	29.1	85.3	175.0
pea protein isolate	66.0	42.6	92.3	145.0
gelatin	5.0	15.3	19.1	100.0
sunflower protein isolate	47.0	31.0	105.8	230.0
rapeseed protein isolate	55.0	44.0	66.2	140.0
whey protein	182.0	88.7	52.2	220.0
bovine serum albumin	325.0	100.0	25.0	340.0
β-lactoglobulin	426.0	100.0	4.2	210.0

[a] Average of duplicate determinations. [b] I: determined according to the new method described here. II: determined according to the method of Wang and Kinsella (1976). [c] %: mL of oil/100 g of sample (dry weight).

metaphosphate ion was linked to the positive (amino) groups of the protein by a saltlike bond of very low dissociation tendency (i.e., ionizing capacity). Thus, when the solution containing protein and metaphosphate was dialyzed at pH >7, the metaphosphate was readily removed and the protein was obtained with all of its original properties unchanged (Briggs, 1940). The protein–metaphosphoric acid complex was also easily solubilized by salt addition (Perlmann and Herrmann, 1938). The extent of protein denaturation by metaphosphoric acid should be minimum since the FBC values obtained for proteins of low solubility did not change when water replaced metaphosphoric acid in the procedure. The protein loss was less than 10%. For insoluble proteins, the correction for protein loss is negligible.

After homogenizing and holding the protein sample for 30 min (to allow the air bubbles to rise to the surface), some proteins (e.g., soy protein) formed a thin usually broken foam layer floating on the sample surface. In this case, an aliquot was taken for absorbance measurement by dipping a Pasteur pipet through a hole of the foam layer into the sample dispersion. If the foam layer was not well broken, the beaker containing the sample dispersion was slightly inclined. Thus, the layer was moved to a direction opposite to that of the inclination, allowing the taking of an aliquot without disturbing the foam layer. The formation of the foam layer was due to the large volume of the homogenizer's container (100 mL) relative to that of the sample (20 mL), allowing the incorporation of air into the sample. Its formation, therefore, may be avoided by using a smaller container (e.g., 25 mL if available).

Comparison of the FBC of Various Proteins. Fat absorption of proteins is affected by the protein source, extent of processing and/or composition of protein, particle size, and temperature (Hutton and Campbell, 1981). The FBC values for some food proteins are given in Table I. As is seen, soy protein isolate and sunflower protein isolate bound more oil than the other proteins tested. On the other hand, β-lactoglobulin bound the least amount of oil among all the proteins tested. The FBC of the proteins in Table I was determined by using the equation derived from the standard curve without any correction, since the reagent blank (protein plus digestion medium) of different proteins had an absorbance value very close to 0.025 (absorbance of reagent blank of soy protein isolate used as a standard protein for construction of the standard curve). In the case of rapeseed protein isolate and Promine D

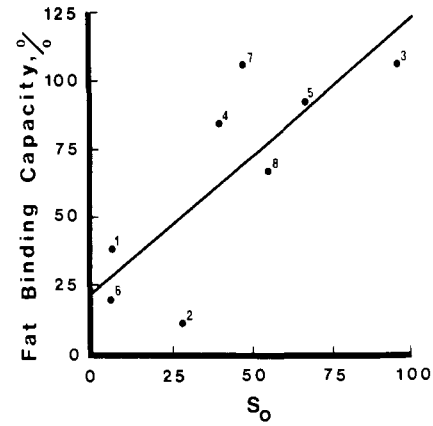

Figure 5. Relationship between hydrophobicity (S_0) and fat binding capacity of food proteins. 1, ovalbumin; 2, casein; 3, soy protein isolate; 4, Promine D; 5, pea protein isolate; 6, gelatin; 7, sunflower protein isolate; 8, rapeseed protein isolate.

(another commercial soy protein isolate), however, since their reagent blanks had absorbance values of 0.9 and 0.05, respectively, a correction was made to compensate for these excessive blank (>0.025) absorbances. Thus, 0.065 (0.09–0.025) and 0.025 (0.05–0.025) were subtracted from the absorbances observed for rapeseed and Promine D, respectively, and then, these net absorbance values were entered into the equation of the standard curve for FBC determination.

Table I also includes the FBC values of the same food proteins determined by the method of Lin et al. (1974) after its slight modification by Wang and Kinsella (1976). It is noteworthy that ovalbumin and β-lactoglobulin had very high FBC values while the present method yielded considerably lower values. In general, the values determined by the method of Wang and Kinsella (1976) were considerably greater than the values measured by the new method, probably because the former method measured mainly the amount of oil physically entrapped as claimed by Wang and Kinsella (1976), whereas in the latter method, the entrapped oil was eliminated as much as possible, thus measuring the amount of truly bound oil.

Statistical Analysis. Regression analysis was used to quantify the relationship between FBC of 11 food proteins and various independent variables. Simple linear regression analysis showed no significant correlation between S_0 and FBC. Multiple linear regression analysis of S_0, s, and FBC also did not show any significant correlation. However, when simple linear regression analysis was applied to correlate S_0 and FBC of only eight proteins of Table I (i.e., β-lactoglobulin, BSA, and whey protein were excluded), the coefficient of determination was significant ($r^2 = 0.619$, $p < 0.05$). The regression equation was FBC (%) = 22.78 + 0.9976S_0 with an S_{xy} of 22.21. This correlation can be seen in Figure 5.

Meanwhile, backward stepwise regression analysis between FBC and various independent variables showed a highly significant correlation between S_0, interaction of S_0 and s (i.e., $S_0 \times s$), and FBC ($R^2 = 0.802$, $P < 0.01$). A multiple regression model for prediction of S_0 and ($S_0 \times s$) effects on FBC is presented in Table II. FBC was positively affected by S_0 whereas the interaction of S_0 with solubility had a negative effect on it. The β values (normalized coefficients) in this model suggest that both S_0 and ($S_0 \times s$) were equally important in determining the FBC of these proteins. The r^2 (coefficient of determination) and R^2 (coefficient of multiple determination) values indicate the percentage of variation in a dependent variable accounted for by its regression on the independent variable

Table II. Multiple Regression Model for Prediction of Fat Binding Capacity of Various Food Proteins[a]

dependent variable	variable description	regression coefficient	F ratio	F probability	β value
fat binding	S_0	1.381	21.77	0.002	5.034
capacity (R^2 = 0.802)	$S_0 \times s$	−0.014	25.91	0.001	−5.492
standard error of estimate = 19.01 (F probability = 0.0015)	constant	30.271	8.41	0.020	0.793

[a] n = 11.

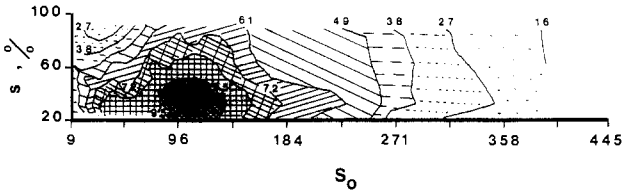

Figure 6. Fat binding capacity response surface contour as a function of hydrophobicity (S_0) and solubility index (s).

or variables, respectively. Comparing simple and multiple regression models in terms of their ability to more accurately predict the FBC of proteins, it is obvious that the latter is the model of choice, since 80.17% of the variation in FBC could be accounted for by the multiple regression model of Table II (as opposed to 61.91% of the variation in FBC of only eight proteins accounted for by the simple regression model). Figure 6 shows the response surface contour of the FBC (of the 11 food proteins of Table I) as a function of S_0 and solubility index. The isoresponse line with the highest FBC value corresponds to relatively medium S_0 (75–125) and low solubility (20–48%) values. As S_0 decreased below or increased above these values, the FBC decreased. All the above results, therefore, suggest that S_0 plays a very important role in the fat binding process.

It should be noted that no correlation (simple or multiple of any form) was found for FBC of the same food proteins determined by the method of Wang and Kinsella (1976) with S_0 or S_0 and s.

The finding by this study that high protein solubility negatively affected the FBC of proteins has some resemblance with the results of the work of Torgersen and Toledo (1977), who correlated physical properties of proteins with their functional characteristics in comminuted meat systems. They found a significant positive correlation coefficient between solubility and fat binding (fat release on cooking), which meant that the more soluble the protein additives the lesser the fat binding properties of the system to which these protein preparations were used. Dippold (1961) reported that a doughnut mix containing 4% soy flour of high solubility (NSI of 80%) absorbed about 50% less fat than the same mix containing 4% soy flour of low solubility (NSI of 60%). Although the aforementioned studies were conducted on complex food systems and the fat binding capacity was determined differently (as fat release or absorption on cooking), and therefore a direct comparison with the finding in this study may be inappropriate, it is likely that high solubility of proteins has an adverse effect on their fat binding capability. One possible reason for the adverse effect of high solubility on the FBC of proteins observed in this study is the conformation of the soluble proteins (BSA, β-lactoglobulin, and whey protein) which does not permit their binding sites (hydrophobic side chains) to be sterically available for interaction with oil hydrocarbon chains. This explanation is supported by the fact that BSA, β-lactoglobulin (Pham, 1981), and whey proteins (Morr, 1979) have mainly α-helical conformation as opossed to the random of β-pleated sheet conformation of soy protein (Wolf, 1972). Another reason may be a limited access of oil to hydrophobic sites of soluble (100%) proteins due to the presence of an excessive number of polar groups forming a barrier around the surface hydrophobic groups of protein.

The finding of this study that with increasing S_0 the FBC is increased and subsequently decreased (at high S_0 values) may be explained by taking into consideration the suggestion of Wolf and Cowan (1975) that fat absorption may be another aspect of emulsification, since in ground meat products fats absorption or binding appears to involve formation and stabilization of an emulsion. According to Aoki et al. (1981), however, the emulsifying properties of proteins ultimately depend on the suitable balance between hydrophile and lipophile and do not necessarily increase as the proteins become more lipophilic. These situations are similar to the concept of the required HLB (hydrophile–lipophile balance) values of fats and the HLB values of surfactants for emulsification.

Mechanism of Fat Absorption. Factors effecting the protein–lipid interaction include protein conformation, protein–protein interactions, and the spatial arrangement of the lipid phase resulting from the lipid–lipid interaction (Hutton and Campbell, 1981). Noncovalent bonds, such as hydrophobic, electrostatic, and hydrogen, are the forces involved in the protein–lipid interactions. Hydrogen bonding is of secondary importance in lipid–protein complexes, although it is indirectly important in hydrophobic bonding (Karel, 1973), since in aqueous media the water–water interactions by hydrogen bonding is much stronger than the interaction between water and nonpolar groups, thus giving rise to hydrophobic bonding beteen nonpolar groups. Electrostatic attraction can occur between the negatively charged phosphate groups of phospholipids and positively charged protein groups (such as lysyl or guanidyl) or between a positively charged group in the phospholipid (e.g. choline) and a negatively charged amino acid side chain (e.g., aspartyl). A related mode of binding is the formation of salt bridges between a negatively charged amino acid side chain and a negatively charged phosphate group of a phospholipid via divalent calcium or other metal ions (Karel, 1973; Pomeranz, 1973; Ryan, 1977). Hydrophobic bonding is likely to play a major role in stabilizing the interactions of both polar and nonpolar lipids with proteins (Ryan, 1977). Moreover, nonpolar dispersion or van der Waals forces become important when interacting groups are near (Karel, 1973) and may play a role in attraction between nonpolar groups in systems in which hydrophobic interaction is unlikely because of limited water (Pomeranz, 1973).

As with the protein–protein interactions, it is not possible to attribute protein–lipid interactions to any single specific kind of molecular force (Ryan, 1977). However, according to Wall (1979) lipids bind to proteins mainly through association with hydrophobic groups. In the method described in this study electrostatic attraction does not seem to play any role in lipid–protein interaction, since the oil used was refined and so should have a negligible amount of phospholipids. The fact that a highly significant coefficient of determination (R^2 = 0.802) was observed between S_0, $S_0 \times s$, and FBC of the food proteins tested

suggests that S_0 is a major determinant of FBC of proteins.

LITERATURE CITED

Aoki, H; Taneyama, D.; Orimo, N.; Kitagawa, I. *J. Food Sci.* **1981**, *46*, 1192.
Brewer, J. M.; Pesce, A. J.; Ashworth, R. B. "Experimental Techniques in Biochemistry"; Prentice-Hall: Englewood Cliffs, NJ, 1974.
Briggs, D. R. *J. Biol. Chem.* **1940**, *134*, 261.
Dippold, M. W. "What's New in Products from the Soybean", Midwest Section American Association of Cereal Chemists Symposium, 1961 (Cited by Johnson, D. W. *J. Am. Oil Chem. Soc.* **1970**, *47*, 402).
Franzen, K. Ph.D. Thesis, Cornell University, 1975 (Cited by Kinsella, J. E. *CRC Crit. Rev. Food Sci. Nutr.* **1976** *7*, 219).
Hutton, C. W.; Campbell, A. M. In "Protein Functionality in Foods"; Cherry, J. P., Ed.; American Chemical Society: Washington, DC, 1981; ACS Symp. Ser. No. 147, p 177.
Johnson, D. W. *J. Am. Oil Chem. Soc.* **1970**, *47*, 402.
Karel, M *J. Food Sci.* **1973**, *38*, 756.
Kato, A.; Nakai, S. *Biochim. Biophys. Acta* **1980**, *624*, 13.
Kinsella, J. E. *Food Technol. (Chicago)* **1975**, *29* (5), 82.
Kinsella, J. E. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *7*, 219.
Lin, M. J. Y.; Humbert, E. S.; Sosulski, F. W. *J. Food Sci.* **1974**, *39*, 368.
Morr, C. V. In "Functionality and Protein Structure"; Pour-El, A., Ed.; American Chemical Society: Washington, DC, 1979; ACS Symp. Ser. No. 92, p 65.
Morris, C. E. *Food Eng.* **1980**, *52* (9), 28.
Nakai, S.; Ho, L.; Tung, M. A.; Quinn, J. R. *Can. Inst. Food Sci. Technol. J.* **1980**, *13*, 14.
Nakai, S.; Le, A. C. *J. Dairy Sci.* **1970**, *53*, 276.
Pearse, K. N.; Kinsella, J. E. *J. Agric. Food Chem.* **1978**, *26*, 716.
Perlmann, G. *Biochem. J.* **1938**, *32*, 931.
Perlmann, G.; Herrmann, H. *Biochem. J.* **1938**, *32*, 926.
Pham, A.-M. M.Sc. Thesis, University of British Columbia, Vancouver, B.C., Canada, 1981.
Pomeranz, Y. *Adv. Food Res.* **1973**, *20*, 153.
Ryan, D. S. In "Food Proteins: Improvement through Chemical and Enzymatic Modifications"; Feeney, R. E.; Whitaker, J. R., Eds.; American Chemical Society: Washington, DC, 1977; ACS Adv. Chem. Ser. No. 160, p 67.
Torgersen, H.; Toledo, R. T. *J. Food Sci.* **1977**, *42*, 1615.
Voutsinas, L. P.; Cheung, E.; Nakai, S. *J. Food Sci.* **1982**, in press.
Wall, J. S. *Cereal Foods World* **1979**, *24* (7), 288.
Wang, J. C.; Kinsella, J. E. *J. Food Sci.* **1976**, *41*, 286.
Wolf, W. J. In "Soybeans: Chemistry and Technology"; Smith, A. K.; Circle, S. J., Eds.; Avi Publishing Co.: Westport, CT, 1972.
Wolf, W. J.; Cowan, J. C. "Soybeans as a Food Source"; CRC Press: Cleveland, OH, 1975.

Received for review May 11, 1982. Accepted September 24, 1982. A part of this work was presented at the 24th Annual Conference of the Canadian Institute of Food Science and Technology, Winnipeg, Manitoba, Canada, June 21–24, 1981.

A Gas–Liquid Chromatographic Method for the Analysis of Methionine and of Methionine Sulfoxide in Proteins

Klaas D. Bos, Cornelis Verbeek, and Pier Slump*

The determination of methionine and methionine sulfoxide in feeds by gas–liquid chromatography is described. Methionine is determined as methyl thiocyanate, after reaction of the intact protein with cyanogen bromide. The indirect determination of the methionine sulfoxide involves the reduction of the sulfoxide with titanium trichloride and subsequent determination of the methionine via the cyanogen bromide method. The difference between this value for methionine and that obtained via the direct determination of the methionine without a reduction step gives the amount of methionine sulfoxide. The method was applied to a series of feed and food proteins. Some of these samples had a sulfoxide content of 10–20% of total methionine. This technique has potential for measuring nutritionally available methionine in feeds by a rapid chemical method, which does not require protein hydrolysis.

Methionine is an essential amino acid that is the limiting factor in several feed proteins (Woodham, 1978). Part of the methionine in certain proteins may be present in an oxidized form, often the sulfoxide, but, in strongly oxidized proteins, methionine sulfone may be present also. In recent years, several papers have been published on the biological availability of methionine in relation to the presence of methionine sulfoxide and sulfone (Slump and Schreuder, 1973; Gjøen and Njaa, 1977; Kuzmicky et al., 1977; Sjöberg and Boström, 1977; Cuq et al., 1978; Ellinger, 1978). Methionine sulfone has been found to be biologically nonavailable (Kuzmicky et al., 1977; Sjöberg and Boström, 1977), and the availability of methionine sulfoxide is generally expected to be less than that of methionine (Gjøen and Njaa, 1977; Kuzmicky et al., 1977; Cuq et al., 1978). A good analytical method for the determination of methionine sulfoxide is of great value for the interpretation of the results of feeding experiments. Methionine sulfoxide in proteins has been determined by two *direct* methods, one involving alkaline hydrolysis and determination of the methionine sulfoxide by automatic amino acid analysis (Neumann, 1967) and the other involving a reaction with acetic anhydride and determination of the formaldehyde formed by a color reaction with chromotropic acid (Lunder, 1972).

Alternatively, some *indirect* methods may be used to determine methionine sulfoxide in proteins. For example, reaction of a protein with iodoacetic acid will convert the methionine residues to sulfonium salts. Subsequent oxidation with performic acid results in the formation of methionine sulfone from the methionine sulfoxide, if present. Upon hydrolysis the methionine sulfone is determined by ion-exchange chromatography (Neumann, 1967; Slump and Schreuder, 1973; Sjöberg and Boström, 1977). The direct method, involving alkaline hydrolysis, may result in low recoveries of methionine sulfoxide (Neumann, 1967; Lunder, 1972). The indirect method with iodoacetic acid is complicated and time consuming and is

Institute CIVO-Analysis TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

0021-8561/83/1431-0063$01.50/0