adopted to routinely predict forage quality with high accuracy.

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LITERATURE CITED

Barton, F. E., II, Amos, H. E., Burdick, D., Wilson, R. L., *J. Anim. Sci.* **43**, 504 (1976).
Barton, F. E., II, Burdick, D., data from set of 28 bermuda grass samples on FQA-51, 1977a.
Barton, F. E., II, Burdick, D., same data set, samples run on Neotec spectrocomputer of G. S. Birth, 1977b.
Ben Gera, I., Norris, K. H., *Israel J. Agric. Res.* 18, 125 (1968).
Hymowitz, T., Dudley, J. W., Collins, F. E., Brown, C. M., *Crop Sci.* 14, 713 (1974).
Kaye, W., *Spectrochim. Acta* 6, 257 (1954).
Kaye, W., *Spectrochim. Acta* 7, 181 (1955).
Norris, K. H., Hart, J. R., *Humidity Moisture; Meas. Control Sci. Ind., Pap. Int. Symp. 1963* 4, 19 (1965).
Norris, K. H., Barnes, R. F., Moore, J. E., Shenk, J. S., *J. Anim. Sci.* **43**, 889 (1976).
Norris, K. H., Barnes, R. F., "Infrared Reflectance Analysis of Nutritive Value of Feedstuffs", First International Symposium, Feed Composition, Animal Nutrient Requirements, and Computerization of Diets, Fonnesbeck, P. V., Harris, L. E., Kearl, L. C., Ed., Utah Agr. Exp. Sta., Utah State University, Logan, Utah, 1976.
Rinne, R. W., Gibson, S., Bradley, J., Seif, R., Brim, C. A., *Agric. Res. Pub.* ARS-NC-26, USDA (1975).
Shenk, J. S., Department of Agronomy, Pennsylvania State University, State College, PA, personal communication, 1977a.
Shenk, J. S., Department of Agronomy, Pennsylvania State University, State College, PA, personal communication of results on similar samples, 1977b.
Shenk, J. S., Barnes, R. F., Proceedings of the 34th Southern Pasture and Forage Crop Improvement Conference, Auburn, AL, 1977.
Shenk, J. S., Mason, W. N., Risius, M. L., Norris, K. H., Barnes, R. F., Application of Infrared Reflectance Analysis to Feedstuff Evaluation, "First International Symposium, Feed Composition, Animal Nutrient Requirements, and Computerization of Diets", Fonnesbeck, P. V., Harris, L. E., Kearl, L. C., Ed., Utah Agr. Exp. Sta., Utah State University, Logan, Utah, 1976.
Shenk, J. S., Hoover, M. R., Infrared Reflectance Spectro-computer Design and Application Advances in Automated Analysis, "Technicon International Congress", Vol. 2 Industrial Symposia, New York, NY, Dec. 13–15, 1976.
Shenk, J. S., Norris, K. H., Barnes, R. F., Forage and Feedstuff Analysis with Infrared Reflectance Spectro-computer System, Proceedings of the 13th International Grasslands Congress, Leipzig, German Democratic Repubic, 1977.
Shenk, J. S., Westerhaus, M. O., Hoover, M. R., Infrared Reflectance Analysis of Forages, Proceedings International Grain and Forage Harvesting Conference, American Society of Agricultural Engineers, St. Joseph, MI, 1978.

Received for review March 6, 1979. Accepted June 6, 1979. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others which may be suitable.

Industrial Whole Animal Blood. Characterization Studies and Quantitative Protein Removal by Chemical Coagulation

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Industrial whole animal blood samples were obtained from processors and analyzed for total nitrogen, volatile nitrogen, nonprotein nitrogen, and ammonia nitrogen levels by Kjeldahl analysis. Blood samples obtained from a renderer displayed greater protein decomposition than samples obtained from a packer. Whole blood samples were treated, under conditions of low dilution and at acidic pH values, with the protein coagulants sodium polyphosphate, ferric chloride, lignin, or sodium lignosulfonate. Sodium polyphosphate or ferric chloride quantitatively removed blood protein, while lignin or sodium lignosulfonate treatment resulted in near quantitative protein removal, under the conditions tested. The potential use of chemical coagulants for industrial reclamation of blood proteins is discussed.

Protein derived from animal blood is reclaimed by the meat packing and rendering industries and is used to supplement animal feed products (Waibel et al., 1977). Blood protein may be reclaimed quantitatively by cooking whole blood to dryness, or nonquantitatively by steam coagulation of whole blood, followed by separation of the coagulated material from the resulting effluent. Each of these methods creates problems for the meat packer and renderer. In the case of whole blood drying (vat drying), the high water content of blood necessitates a large investment in heat energy to dry the product. In addition, vat drying results in severe reduction in the levels of lysine, methionine, and cystine, as well as diminished digestibility of the protein product (Waibel et al., 1977; Kramer et al., 1978). Steam coagulation of blood, followed by separation of the coagulated solids, often results in nonquantitative removal of protein from blood producing an effluent serum which is high in biochemical oxygen demand (BOD). The serum effluent contains nitrogen, in the form of colloidal protein and suspended solids, as well as soluble nonprotein containing compounds. The most common approach to the serum effluent problem is to develop coagulation–flocculation techniques for removal of protein from the effluent before it leaves the processor as sewage (Hopwood and Rosen, 1972; Sanders, 1948).

A different approach to the recovery of blood protein lies in the development of techniques to remove the protein directly from blood using chemicals. Chemical coagulation of blood protein could be competitive with conventional methods if: (1) the chemical treatment did not require heat or diminished the amount of heat currently used; (2) the chemicals were cost competitive; (3) the chemicals were nontoxic or nutritionally desirable; (4) little residual chemicals were released in the effluent produced; (5) the nu-

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tritional quality of the resultant product was enhanced; and, (6) the removal of the protein was essentially quantitative. We have investigated the use of the nontoxic protein coagulants sodium polyphosphate, sodium lignosulfonate, lignin, and ferric chloride for effectiveness of protein removal from blood. These chemicals have been used by others for removal of protein from the wastewaters of cheese processors (Jones et al., 1972; Block and Bolling, 1955; Cerbulis, 1978; Hartman and Swanson, 1966; Riehert, 1973; Hidalgo et al., 1973), slaughter houses (Herstad and Hvidsten, 1973; Sanders, 1948; Hopwood and Rosen, 1972), and fish processors (Claggett and Wong, 1969; Spinelli and Koury, 1970). Our results demonstrate that chemical removal of blood protein, as a primary step in blood treatment, is feasible since several of the above discussed criteria are satisfied by such a process.

EXPERIMENTAL SECTION

Materials and Methods. The coagulant Politol NA (lignin), a lignin compound produced by alkaline wood pulping, was obtained from Westvaco Chemical Co. (Charleston, SC). PRA no. 1 (SLS), a lignosulfonate produced by sulfite wood pulping, was obtained from the American Can Co. (Greenwich, CT). Sodium polyphosphate (sodium hexametaphosphate, average chain length = 15, SPP) was obtained from Sigma Chemical Co. (St. Louis, MO). Anhydrous ferric chloride, reagent grade, was obtained from Fisher Scientific Co. (Fair Lawn, NJ). All other chemicals were reagent grade and were obtained from local suppliers. Fresh whole blood (bovine and porcine) was obtained from a local slaughterhouse. Industrial whole animal blood samples, held no longer than 4 h past slaughter, were obtained from the holding tanks of the Emge Packing Co. (Fort Branch, IN) and frozen at −20 °C. Control experiments were performed using blood samples from the same source, which differed only as to whether or not they had undergone freezing and thawing prior to coagulant treatment. These experiments demonstrated that freezing and thawing of blood had no effect on the protein-removing ability of the coagulants tested. Storage at −20 °C for up to 3 months did not affect the quality of the blood. Whole blood samples were also obtained from a renderer (A. W. Stadler Inc., Cleveland, OH). Industrial blood samples occasionally contain some clotted material. Clots were removed by filtration through cheesecloth, followed by characterization of the blood as described below.

Blood samples were analyzed for Kjeldahl nitrogen using a modification of a technique described in the 13th edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association, 1971). Volatile nitrogen was determined by removal of nitrogen from samples adjusted to pH 7.4 with phosphate buffer. Duplicate samples of those used for volatile nitrogen determinations were used for total Kjeldahl nitrogen determinations. The volatile nitrogen level was subtracted from the total Kjeldahl nitrogen level to obtain organic nitrogen levels.

Nonprotein nitrogen was determined by quantitative precipitation of protein using 10% trichloroacetic acid (Cl_3CCOOH), followed by analysis of the centrate for total Kjeldahl nitrogen. Nonprotein nitrogen values represent a measurement of urea, ammonia, amino acids, and other nonprecipitable nitrogen-containing compounds. On the other hand, volatile nitrogen values represent a measurement of only those nitrogen compounds which are volatile at pH 7.4.

Free ammonia in blood was analyzed by a modified method of Van Slyke and Cullen (Bray, 1951). Protein was removed by precipitation using 10% Cl_3CCOOH, and the centrate was made alkaline (pH 13.5) and aerated to remove ammonia. The ammonia was collected by passing the air stream through a 2% boric acid solution and titrated with standard H_2SO_4. Total carbon analyses were performed using a Beckman Model 915 total organic carbon analyzer. Total carbon levels can be directly correlated with COD, BOD, and protein remaining in solution after chemical coagulant treatment (Chandler et al., 1976).

Table I. Analysis of Whole Blood[a]

sample	volatile nitrogen	total nitrogen	nonprotein nitrogen	NH_3 nitrogen
1	120	27 100	np[b]	np
2	110	35 200	np	np
3	110	26 000	np	np
4	60	27 500	280	np
5	2900	18 500	3700	2750
6	3000	29 000	3300	840
7	260	30 000	500	80
8	250	31 000	390	70
9	390	30 750	470	90

[a] All values are in milligrams of N/liter. Samples 1–4 were obtained from a renderer while samples 5–9 were obtained from a packer. [b] Not performed.

Protein coagulation was effected by adding the coagulant as a solid or in solution to blood diluted with doubly distilled water by a dilution factor of about 1:2. SPP (added as a solid), SLS, or lignin (each added as 10% water solutions) were next added to the diluted blood, followed by adjustment of the pH to the desired value with 0.5 M H_2SO_4. In all experiments using SPP, SLS, or lignin the final dilution used was 1:3. When $FeCl_3$ was used as coagulant, blood was diluted by a factor of about 1:4 with doubly distilled water prior to addition of $FeCl_3$ (added as a 10% solution), and adjustment of the pH with 0.5 M NaOH. In all experiments using $FeCl_3$ the final dilution used was 1:5. Optimal pH values for each coagulant were determined by coagulation experiments performed using near optimal amounts of a given coagulant at various pH values. The addition of concentrated acid to diluted blood causes clumping which interferes with coagulation. Therefore, as long as low dilution levels of blood are used in coagulant treatment, diluted acid is necessary for pH adjustment. All coagulation experiments were performed at room temperature (25 °C), with 1-h settling times prior to centrifugation at 3000*g* for 10 min and analysis of the centrate. Phosphate analyses were performed by the vanadomolybdophosphoric acid method (American Public Health Association, 1971) after acid hydrolysis of the polyphosphate.

RESULTS AND DISCUSSION

Blood obtained from industrial sources is largely unclotted although it may contain varying amounts of suspended clots. Many packers agitate their blood samples either mechanically during collection, or indirectly by allowing the blood to flow to a holding tank. The constant flow or agitation of blood, perhaps coupled with cooling, is responsible for the diminished clotting observed in industrial blood samples.

Data summarizing the nitrogen content of nine whole blood samples are displayed in Table I. Four of the samples (1–4) represent fresh whole blood obtained locally from porcine and bovine species. Samples numbered 5 and 6 were obtained from renderers while samples numbered 7, 8, and 9 were obtained from a packing house. Fresh whole blood is characterized by low volatile nitrogen (60–120 mg of N/L) and low nonprotein nitrogen levels (250–350 mg of N/L) relative to the total nitrogen content (~31 000 mg of N/L). Volatile nitrogen and nonprotein

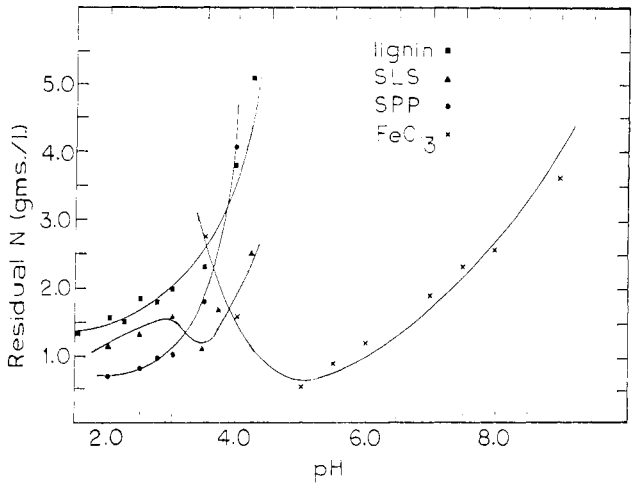

Figure 1. Normalized residual total Kjeldahl nitrogen after coagulant treatment of diluted blood vs. pH. Treatment conditions were as described in the text. Final coagulant concentrations were SPP (6.7 g/L), $FeCl_3$ (19 g/L), SLS (22 g/L), and lignin (20 g/L).

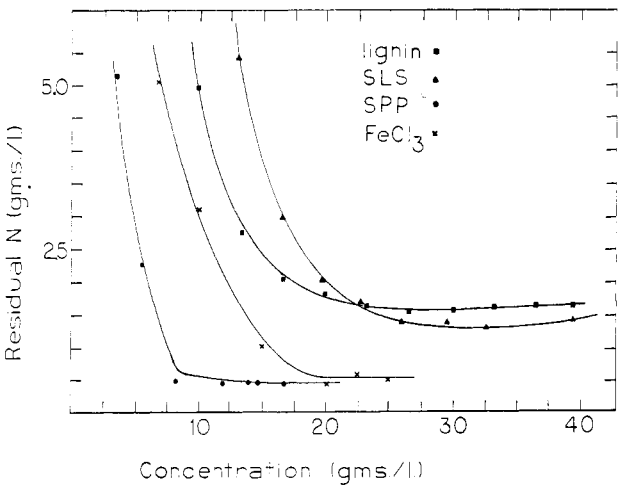

Figure 2. Normalized residual total Kjeldahl nitrogen after treatment of diluted blood using various coagulants versus coagulant dosage. See text for experimental details.

nitrogen values for blood samples obtained from a packer are typically on the order of twice that observed for fresh blood, but are still relatively low. However, the fact that ammonia levels of blood obtained from the packer are almost as high as volatile nitrogen values of fresh blood suggests that some protein decomposition has occurred. Blood samples obtained from renderers are high in volatile and nonprotein nitrogen levels and in ammonia content. Nonprotein nitrogen levels are seven–eight times greater and ammonia levels are at least ten times greater in blood samples obtained from a renderer as compared to those from a packer.

In the development of protein coagulation techniques, the low nonprotein nitrogen levels of fresh whole blood represent the lowest achievable level of residual nitrogen in effluents. Nonprotein nitrogen components in blood are low-molecular-weight compounds which cannot be removed from blood by steam or chemical coagulation methods. [For a description of blood processing techniques, see Kramer et al. (1978).] Regardless of the protein recovery process used, greater blood decomposition represents a loss of recoverable protein in the form of low-molecular-weight compounds.

Data summarizing the effectiveness of SPP (sodium hexametaphosphate), lignin, $FeCl_3$, and SLS for protein removal from blood are displayed as Figures 1 and 2. Each data point for each figure represents an average of at least three independent experiments using blood samples obtained from a packer where the nonprotein nitrogen levels were never greater than 500 mg/L, while the total carbon levels were approximately 105 000 ppm. When using SPP, lignin, or SLS, the final volume of blood represents a dilution of three times the original volume. When $FeCl_3$ is used as a coagulant, the final volume is five times the original volume of untreated blood. Some dilution of whole blood during coagulation proved to be necessary, especially due to solubility difficulties involving coagulants which are added as solids (SPP). Also, compounds such as $FeCl_3$ cause localized coagulation if added in too great a concentration (even if added as a solution) which impedes the effectiveness of the additional coagulant needed for effective protein removal. Since dilution is necessary, it is reasoned that the optimal dilution value would be the same as the dilution of blood resulting from steam coagulation treatment, since steam coagulation is commonly used to remove blood proteins. We estimate that blood might be diluted to as much as three times its original volume during steam coagulation procedures. Although our calculated dilution level is somewhat arbitrary, the level at least seems to be a reasonable starting point, since the true dilution value is not known and is subject to a variety of local conditions (W. Van Steenberg, De Laval Co., Inc., personal communication, 1979).

In order to take into account the dilution factor in representing data, all values plotted along the ordinates in residual nitrogen and total carbon graphs represent raw data values which have been normalized by multiplication by the dilution factor involved. Multiplication of observed levels allows for a direct comparison of the *total* amount of residual carbon or nitrogen in the effluent relative to the *total* amount of carbon and nitrogen in whole blood. Thus, actual residual total Kjeldahl nitrogen and total carbon values for centrates following coagulant treatment would be one-third the value plotted (one-fifth for $FeCl_3$) but the total volume of centrate would be about three times (five times for $FeCl_3$) that of the original volume of whole blood. Values plotted along the ordinate for residual phosphate are not corrected for dilution since comparison of the total amount of phosphorus in the effluent to that of whole blood is not warranted. Values plotted along the abscissa for all graphs represent actual amounts of coagulants added to the blood *after* it had been diluted to its final treatment volume. Our experimental results suggest that the actual amount of coagulant needed for effective protein removal is determined by the *total* amount of protein present and is independent of the dilution level of the blood.

Experiments designed to determine the most effective pH range for each of the four coagulants were performed by holding constant the amount of coagulant added while varying the pH. As shown in Figure 1, SLS displays a pH optimum of 3.5, $FeCl_3$ displays greatest effectiveness at about pH 4.5, while lignin and SPP display greatest effectiveness in the pH range 2.0 to 3.0.

SPP is extremely effective in the removal of protein from blood. As displayed in Figure 2, at an effective concentration of about 9 g/L (pH 3.0), the total residual nitrogen level of the effluent is very nearly equal to that of the nonprotein nitrogen level of whole blood (500 mg/L). Thus, by this process, SPP removes all potentially removable Kjeldahl nitrogen (about 98.4%) from blood. At the same time, the total carbon level of the blood is reduced from about 105 000 ppm in whole blood to about 2200 ppm

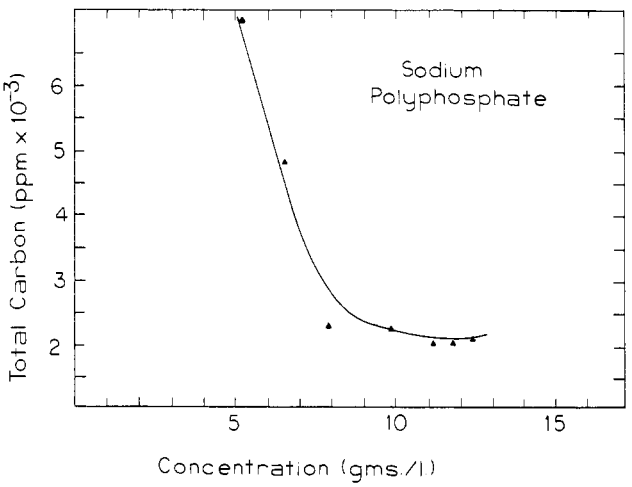

Figure 3. Normalized total residual carbon vs. sodium polyphosphate (SPP) coagulant added to diluted blood at various final concentrations at pH 3.0.

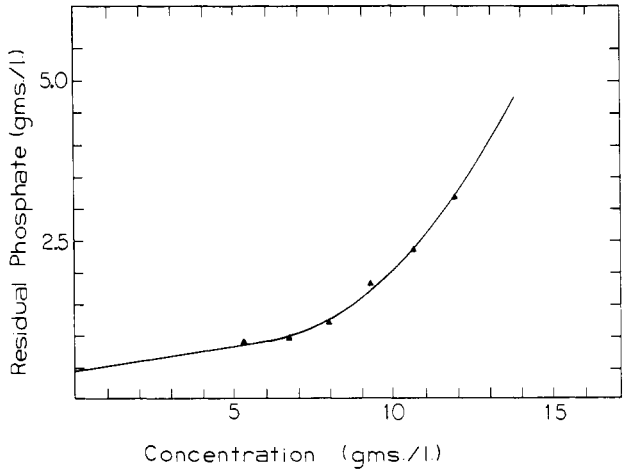

Figure 4. Residual phosphate vs. concentration of sodium polyphosphate (SPP) coagulant added to diluted blood at various final concentrations at pH 3.0.

in the effluent, a 97.8% reduction (Figure 3). As displayed in Figure 4, most of the input phosphate (80–85%) is removed from solution with the coagulated protein–polyphosphate complex. The residual solution phosphate increases rapidly as concentrations of input phosphate higher than the effective concentration are used.

SLS is also very effective in protein removal from blood. At pH 3.5, a 93.1% removal of Kjeldahl nitrogen is observed at a concentration of 22 g of SLS/L (Figure 1). The total carbon level of the blood is reduced from about 105 000 ppm in whole blood to about 12 000 ppm in the effluent; an 89% reduction (Figure 5). The total carbon level in the effluent represents some contribution from the carbonaceous SLS. We estimate that about half of the residual carbon present at the effective concentration of SLS is contributed by the SLS itself.

Lignin is not quite as effective as SLS in removal of protein when used at its optimal concentration and pH (pH 2.0). Lignin removes about 92.7% of the total Kjeldahl nitrogen from blood at the effective concentration of 20 g/L (Figure 1). The residual total carbon value of about 6000 ppm (94% removal) is lower than the residual value observed for SLS (Figure 6). The lower value is due to the fact that lignin has limited solubility at acidic pH and precipitates from solution while SLS does not.

$FeCl_3$, when added to blood, causes localized coagulation of the blood which interferes with the dissolving of enough

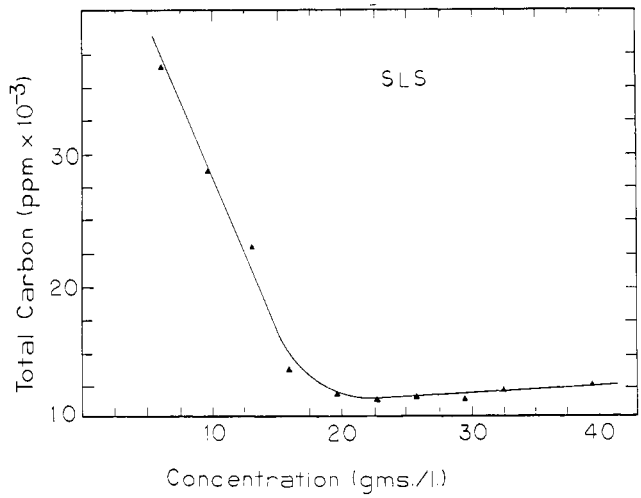

Figure 5. Normalized total residual carbon vs. sodium lignosulfonate (SLS) coagulant added to diluted blood at various final concentrations at pH 3.5.

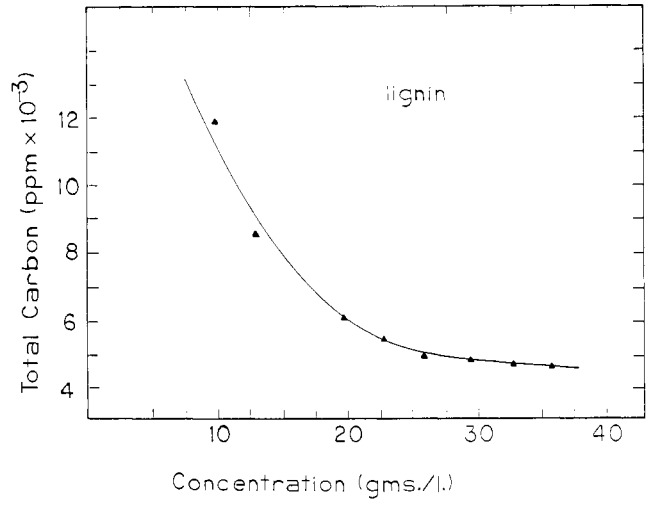

Figure 6. Normalized total residual carbon vs. lignin coagulant added to diluted blood at various final concentrations at pH 2.0.

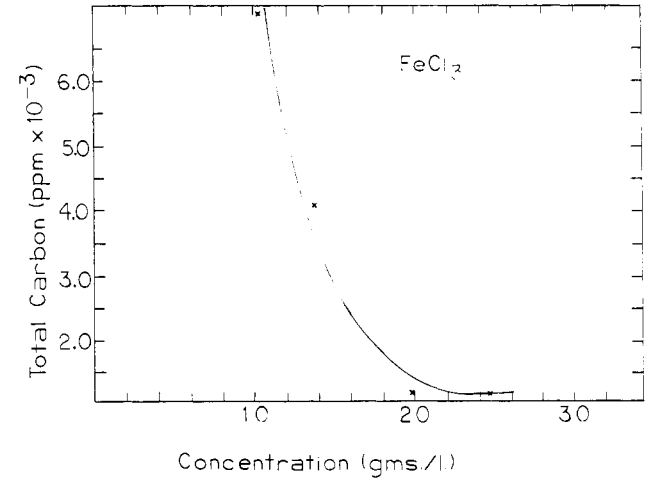

Figure 7. Normalized total residual carbon vs. $FeCl_3$ coagulant added to diluted blood at various final concentrations at pH 4.5.

$FeCl_3$ needed to remove all of the protein. In order to avoid the problem it is necessary to dilute the blood 1:5. At this dilution and at a concentration of about 20 g/L (pH 4.5), $FeCl_3$ removes 98.3% of the Kjeldahl nitrogen (Figure 2) and lowers the total carbon to about 1100 ppm (99.6%) reduction (Figure 7).

Taken together, these data suggest that chemical treatment of blood for reclamation of protein is feasible at the industrial level. Industrial application of chemical coagulation techniques could eliminate or diminish the need for secondary treatment of blood wastewaters since chemical coagulation is capable of quantitative removal of protein in the primary step. We are currently studying other chemical procedures of blood protein removal.

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LITERATURE CITED

American Public Health Association, "Standard Methods for the Examination of Water and Wastewater", 13th ed., Washington, DC, 1971, p 244.
Block, R. J., Bolling, D., to the Borden Co., U.S. Patent 2710858, June 14, 1955.
Bray, W. E., "Clinical Laboratory Methods", C. V. Mosby Co., St. Louis, MO, 1951, p 239.
Cerbulis, J., *J. Agric. Food Chem.* **26**, 806 (1978).
Chandler, R. L., O'Shaugnessy, J. C., Blanc, F. C., *J. Water Pollut. Control Fed.* **48**, 2971 (1976).
Claggett, F. G., Wong, J., Circular No. 42, Salmon Wastewater Clarification Part II, Fish Res. Bd. of Canada, Feb. 1969.
Hartman, G. H., Swanson, A. M., *J. Dairy Sci.* **49**, 697 (1966).
Herstad, O., Hvidsten, H., *Acta Agri. Scandinavica*, **23**, 154 (1973).
Hidalgo, J., Kurseman, J., Bohmen, H. V., *J. Dairy Sci.* **56**, 988 (1973).
Hopwood, A. P., Rosen, G. D., *Proc. Biochem.* **7**, 15 (1972).
Jones, S. B., Kalan, E. B., Jones, T. C., Hazel, J. F., *J. Agric. Food Chem.* **20**, 229 (1972).
Kramer, S. L., Waibel, P. E., Behrends, B. R., El Kandelgy, S. M., *J. Agric. Food Chem.* **26**, 979 (1978).
Riehert, S. M., *Diss. Abstr.* **33**, 3128-B (1973).
Sanders, M. D., *Ind. Eng. Chem.* **6**, 1151 (1948).
Spinelli, J., Koury, B., *J. Agric. Food Chem.* **18**, 284 (1970).
Van Steenberg, W., De Laval Co., Inc., Poughkeepsie, NY, personal communication, 1979.
Waibel, P. E., Cuperlovic, M., Hurrell, R, F., Carpenter, K. J., *J. Agric. Food Chem.* **25**, 171 (1977).

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Determination of the Degree of Hydrolysis of Food Protein Hydrolysates by Trinitrobenzenesulfonic Acid

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An accurate, reproducible and generally applicable procedure for determining the degree of hydrolysis of food protein hydrolysates has been developed. The protein hydrolysate is dissolved/dispersed in hot 1% sodium dodecyl sulfate to a concentration of 0.25–2.5 × 10^{-3} amino equivalents/L. A sample solution (0.250 mL) is mixed with 2.00 mL of 0.2125 M sodium phosphate buffer (pH 8.2) and 2.00 mL of 0.10% trinitrobenzenesulfonic acid, followed by incubation in the dark for 60 min at 50 °C. The reaction is quenched by adding 4.00 mL of 0.100 N HCl, and the absorbance is read at 340 nm. A 1.500 mM L-leucine solution is used as the standard. Transformation of the measured leucine amino equivalents to degree of hydrolysis is carried out by means of a standard curve for each particular protein substrate.

Enzymatically hydrolyzed proteins possess functional properties, such as low viscosity, increased whipping ability, and high solubility, which make them advantageous for use in many food products. Recent experiments have indicated that in order to obtain desirable organoleptic and functional properties of soy protein hydrolysates, the hydrolysis must be carried out under strictly controlled conditions to a specified (generally low) degree of hydrolysis (DH) (Adler-Nissen, 1977; Adler-Nissen and Sejr Olsen, 1979). DH is defined as the percentage of peptide bonds cleaved (Adler-Nissen, 1976). Therefore, a need exists for a general method of determining DH of food protein hydrolysates, in particular for quality control. An obvious method to consider for this purpose is the trinitrobenzenesulfonic acid (TNBS) method, by which the concentration of primary amino groups in the hydrolysate can be determined.

Basically, this method is a spectrophotometric assay of the chromophore formed by the reaction of TNBS with primary amines (Figure 1). The reaction takes place under slightly alkaline conditions and is terminated by lowering the pH. TNBS also reacts slowly with hydroxyl ions, whereby the blank reading increases; this increase is stimulated by light (Fields, 1971).

Since its introduction by Satake et al. (1960), the TNBS method has enjoyed a widespread use for the determination of free amino groups of proteins and protein hydrolysates. However, the presence of insoluble proteinaceous material in, e.g., the commercially used whipping agents based on hydrolyzed soy protein necessitates certain modifications of the various existing procedures described in the literature, as they seem to have been developed for soluble materials only. Also, although it is generally assumed that a linear relationship between the color intensity and the concentration of α-amino groups exists, we have

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