molecular-weight components during gel filtration separation than do urea or GHCl. Since the dissociated proteins are of different molecular weights or are not completely dissociated even in $NaDodSO_4$, they have not been isolated into pure components.

Whether or not these low-molecular-weight fractions separated from glutenin should be classified as glutenin is debatable. The A fraction separated from PE-glutenin is a major contributor to the low-molecular-weight fractions. Thus it appears that the A fraction may not be bonded by intermolecular disulfide links to the B and C fractions (Huebner and Wall, 1974). If it is mainly associated by hydrophobic groups to glutenin, it may be an important factor in gluten aggregation and rapid protein interactions. It has been speculated that this protein is derived from membrane proteins (Simmonds, 1971).

The evidence obtained in these studies still indicates that glutenin is composed of a mixture of components differing in molecular weight. Most of these components are composed of polypeptide chains intermolecularly linked by disulfide bonds to yield chains ranging from 100 000 to 10 million mol wt. From $NaDodSO_4$ patterns of the reduced protein it appears that the different molecular weight fractions vary in subunit composition. The fractions around 100 000 consist mainly of a few C fraction polypeptides. The highest molecular weight fraction consists of the C fractions and the higher molecular weight B subunits, whereas intermediate range fractions contain varying amounts of the lower molecular weight B and C subunits. These results may explain the diversity of glutenin molecular weight and are consistent with previous observations of variations in subunit composition of glutenin solubility fractions (Bietz and Wall, 1973).

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Chemical Coagulation of Industrial Animal Blood Using Aluminum Sulfate, Zinc Sulfate, Methanol, and Acetone

Andrew L. Ratermann, H. Wayne Burnett, and Vaughn Vandegrift*

Industrial whole animal blood samples were treated, under conditions of low dilution, with the protein coagulants aluminum sulfate, zinc sulfate, methanol, or acetone. Aluminum sulfate or methanol treatment resulted in quantitative removal of protein from blood at acidic pH, using the procedure tested. Zinc sulfate also quantitatively removed protein from diluted blood, under basic pH conditions. Acetone was not nearly as effective a protein coagulant as the other reagents tested. The metallic salts used as coagulants produce protein–coagulant complexes which retain significant amounts of water. Similarly, protein coagulated using organic solvents contains significant amounts of solvent. It is concluded that cold blood processing by chemical coagulation holds promise as an economical technique for the production of dried blood suitable for use as an animal feed ingredient.

One of the major byproducts of the meat processing industry is animal blood. Protein is normally reclaimed from animal blood using whole blood drying or steam coagulation techniques (Kramer et al., 1978). Whole blood drying necessitates a large investment in heat energy and results in 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 protein removal and attendant sewage effluent problems (Sanders, 1948). A different approach to blood treatment lies in the development of techniques to remove protein directly from blood by chemical coagulation. We have reported that

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sodium polyphosphate and ferric chloride quantitatively remove protein from animal blood under specified conditions, whereas lignin and sodium lignosulfonate treatment of blood results in near quantitative protein removal (Vandegrift and Ratermann, 1979).

The results reported herein extend our investigation of chemical coagulation methods for protein removal from animal blood. We have used the organic solvents methanol and acetone and the inorganic salts zinc sulfate and aluminum sulfate to effect protein removal from blood. Some of these compounds have been traditionally used clinically to treat whole blood or blood sera. For example, methanol and acetone have been used to precipitate protein from immune sera (Merrill and Fleischer, 1932), whereas zinc chloride in combination with methanol (Folin and Denis, 1912) and zinc salts with alkali have been used to deproteinate blood (Somogyi, 1930) for sugar analysis of the filtrate. Although these methods are effective in the removal of protein from blood, optimal conditions for maximum precipitation are not always used. Since these techniques were not developed for an industrial process, an excess of the precipitant is often used to assure complete removal of protein, optimal pH values are not indicated and the blood is often diluted tenfold during the protein removal procedure. Thus, we have determined for these compounds the optimal conditions of coagulant concentration and pH required to effectively remove protein from moderately dilute industrial blood samples. The results of our investigations suggest the feasibility of the use of the tested compounds as effective agents for industrial cold blood processing.

EXPERIMENTAL SECTION

Materials and Methods. All chemicals used were reagent grade and were obtained from local supplers. Industrial whole animal blood samples, held no longer than 4 h past slaughter, 4 h were obtained from the holding tanks of the Emge Packing Company (Fort Branch, IN) and frozen at −20 °C. Control experiments, performed using blood samples which differed only as to whether or not they had undergone freezing and thawing prior to coagulant treatment, demonstrated that freezing and thawing of blood did not affect experimental results. Storage at −20 °C for up to 3 months did not affect the quality of the blood. Some clots were occasionally present in blood samples and were removed by filtration prior to characterization. Blood samples and centrates were characterized for total Kjeldahl nitrogen, nonprotein nitrogen, and total carbon as described previously (Vandegrift and Ratermann, 1979)

Protein coagulation was effected by adding the coagulant as a solution (zinc sulfate or aluminum sulfate) or as a pure solvent (methanol or acetone) to blood diluted with doubly distilled water by a dilution factor of about 1 to 2. Zinc sulfate heptahydrate was added as a 15% solution, whereas aluminum sulfate 16-hydrate was added as a 20% solution. The pH of the coagulant-blood mixture was adjusted to the desired level with dilute H_2SO_4 or NaOH. In all experiments the final dilution of blood was 1 to 3. All coagulation experiments were performed at room temperature (25 °C), with 1-h settling times prior to centrifugation at 3000*g* and analysis of the centrate. The coagulant–protein complexes were dried at 125 °C for 4 h to determine the percentage of water in the complexes.

RESULTS AND DISCUSSION

Five different blood samples, all mixtures of porcine and bovine blood containing from 50 to 75% porcine blood, obtained from the same source (Emge Co., Fort Branch, IN) were used in coagulation experiments. The samples

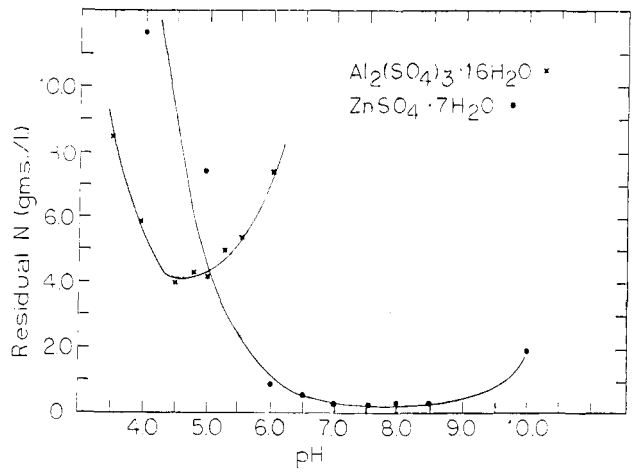

Figure 1. Normalized residual total Kjeldahl nitrogen after coagulant treatment of diluted blood vs. pH. Treatment conditions were as described in the text. Final coagulant concentrations were hydrated aluminum sulfate, 27 g/L, and hydrated zinc sulfate, 10 g/L.

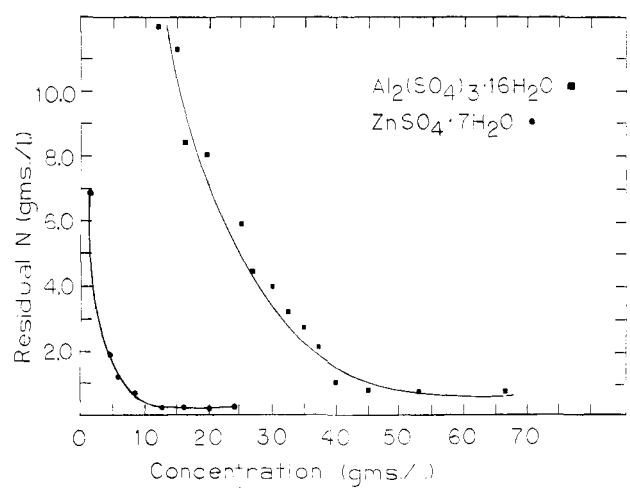

Figure 2. Normalized residual total Kjeldahl nitrogen after treatment of diluted blood vs. the concentration of the coagulants aluminum sulfate (pH 4.5) and zinc sulfate (pH 8.0). Concentrations represent the weight percent of the coagulants as hydrated salts. See text for experimental details.

were fresh blood, as determined by Kjeldahl analysis, since they contained low levels of nonprotein nitrogen (420 ± 50 mg of N/L) relative to the total nitrogen content (2800 ± 200 mg of N/L). Nonprotein nitrogen values represent low-molecular-weight compounds which are not ordinarily removed from blood by steam or chemical coagulation techniques (Vandegrift and Ratermann, 1979). Total carbon levels for the samples used were 100 000 ± 5000 ppm.

Data comparing the effectiveness of the coagulants aluminum sulfate, zinc sulfate, methanol, and acetone for removal of protein from blood is displayed in Figures 1–5. Each data point in all figures represents an average of at least three independent experiments. The final volume of blood in all experiments represents a dilution of three times the original volume. All values plotted along the ordinates in residual nitrogen and total carbon graphs have been normalized by multiplying by the dilution factor. 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 or nitrogen in whole blood. Thus, actual residual total Kjeldahl nitrogen and total carbon values for centrates

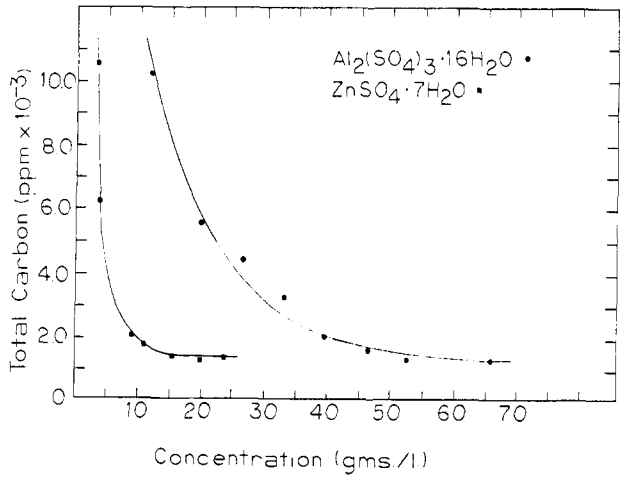

Figure 3. Normalized total residual carbon after treatment of diluted blood vs. aluminum sulfate (pH 4.5) and zinc sulfate (pH 8.0) concentrations. Concentrations of coagulants represent the weight percent of the coagulants as hydrated salts. See text for experimental details.

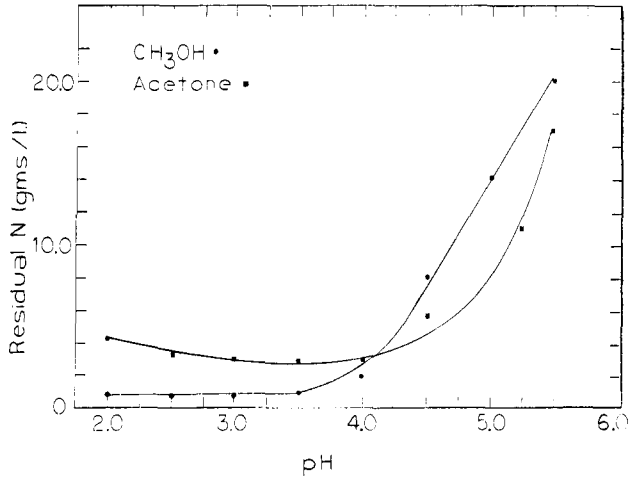

Figure 4. Normalized residual total Kjeldahl nitrogen after treatment of diluted blood vs. pH. Sufficient methanol or acetone was added to produce a final concentration of 20% v/v of the solvents.

following coagulant treatment would be one-third the value plotted, but the total volume of the centrate would be about three times that of the original volume of whole blood. Values plotted along the abscissa represent either the pH to which the diluted blood was finally adjusted (for experiments performed at constant coagulant concentrations) or the actual concentrations of coagulants in the blood after the blood had been diluted to its final treatment volume (for experiments where the final pH was held constant).

Data comparing the effectiveness of the coagulants $Al_2(SO_4)_3 \cdot 16H_2O$ and $ZnSO_4 \cdot 7H_2O$ at various pH values, and at a 1 to 3 dilution of whole blood, is displayed in Figure 1. Aluminum sulfate (27 g/L) displays an optimum at pH 4.5, whereas zinc sulfate (10 g/L) displays an optimum in the range pH 7.5 to 8.5. As shown in Figure 2, at a concentration of 53.0 g/L of hydrated aluminum sulfate, corresponding to a concentration of 28.7 g/L of anhydrous aluminum sulfate, 98.2% of the total Kjeldahl nitrogen is removed from blood diluted 1 to 3. The total carbon level of the effluent is lowered about 98% to 1800 ppm (Figure 3). A comparison of aluminum sulfate to ferric chloride (see Vandegrift and Ratermann, 1979) reveals that the molar concentration of ferric ion is about

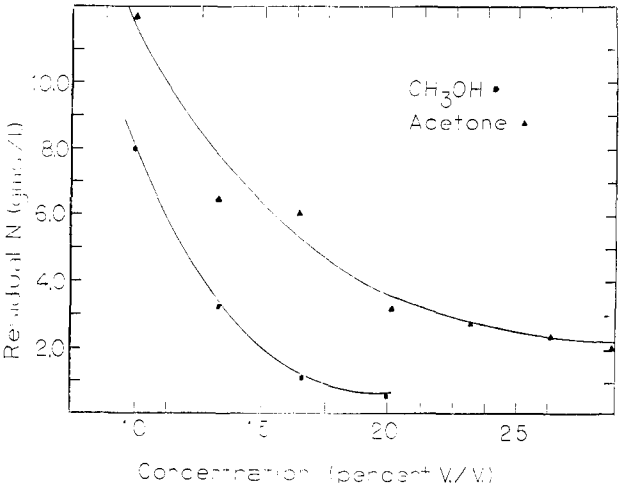

Figure 5. Normalized residual total Kjeldahl nitrogen after treatment of diluted blood vs. methanol or acetone concentration. The pH was adjusted to pH 3.0 (methanol) or to pH 2.5 (acetone) after addition of the coagulant.

the same as the molar concentration of aluminum ion at the effective coagulant dose of each, when the different dilution factors are taken into account. Ferric chloride removes 98.3% of the total Kjeldahl nitrogen at concentration of 20 g/L and at pH 4.5 from blood diluted 1 to 5. Thus, a concentration corresponding to 0.61 mol of ferric ion per liter of undiluted blood and 0.51 mol of aluminum ion per liter of undiluted blood is required to effect quantitative protein removal. The similarity in molar concentration, valence of the ions, and most effective pH of coagulation suggests a similar mechanism for the protein coagulation effected by these ions. Aluminum sulfate may be a more satisfactory compound than ferric chloride due to the lower dilution factor required. However, ferric ion in controlled low quantities as a metal proteinate might be nutritionally desirable in the production of an animal feed.

The coagulation of blood protein using zinc sulfate is essentially quantitative (98%) at 8 g/L in blood diluted 1 to 3 at pH 8.0 (Figure 2). At higher concentrations (20 g/L) zinc sulfate is actually more effective than 10% trichloroacetic acid for protein removal, removing 99.1% of the total Kjeldahl nitrogen. The molar concentration of zinc ion required for quantitative (98%) removal of protein nitrogen is 0.083 mol/L of undiluted blood. Thus, zinc ion at pH 8.0 is significantly more effective than either ferric ion or aluminum ion at pH 4.5. On a weight-to-volume basis, the effectiveness of zinc sulfate (4.5 g/L of dry salt) exceeds that of sodium polyphosphate (9 g/L at pH 3.0) for removal of protein in blood diluted 1 to 3. In addition, the total carbon level of the blood centrate after treatment with 8 g/L of zinc sulfate (2000 ppm, Figure 3) is about the same (98%) as the total carbon level of the centrate following sodium polyphosphate treatment (Vandergrift and Ratermann, 1979).

Although the compounds tested herein, and in our earlier report, are effective coagulants of blood protein, the resulting complexes contain a relatively high water content. For example, the protein complexes produced under the effective coagulant conditions for the salts aluminum sulfate, ferric chloride, and zinc sulfate contain about 83, 80, and 85% water, respectively. Protein complexes produced using sodium polyphosphate, sodium lignosulfonate, and lignin are lower in water content than those produced using inorganic salts but still contain about 70–75% water. The high water content of these complexes would neces-

sitate a great investment of heat energy to produce a dry product.

One approach to lowering the water content of the coagulated protein complexes is to use organic solvents, such as methanol or acetone, to remove the protein. Organic solvents, due to their high vapor pressure, should produce a product which is easy to dry. The resulting dried product would be almost totally composed of protein. In addition, the organic solvent could be recovered from the mother liquor after the coagulation step, or during the drying step, and recycled.

Methanol and acetone each acts as a protein coagulant in diluted blood solutions at acidic pH values. As displayed in Figure 4, methanol has an optimum in the range of pH 2–3, while acetone is effective in the pH range 2.5–3.5 when used at final concentrations of 20% (v/v) in blood diluted 1 to 3. Methanol is significantly more effective than acetone for removal of protein from diluted blood (Figure 5). At a 20% (v/v) concentration in diluted blood (pH 3.0) methanol removes 98.5% of the total Kjeldahl nitrogen, whereas acetone removes 89% of the Kjeldahl nitrogen at a 28% (v/v) concentration (Figure 5). For both the acetone and methanol products 83% of the weight of the complex is lost upon drying.

In spite of the effectiveness of methanol as a blood protein coagulant, the amount of methanol required is significant. Other approaches to lowering the water content of the coagulant–protein complexes are currently being investigated in our laboratory. In addition, we are currently determining the identity and percent of the components present in the coagulant–protein complexes produced by the above described techniques. We believe that cold blood processing by chemical coagulation holds promise as an economical technique for the production of dried blood suitable for use as an animal feed ingredient.

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Substituted 4-Methyl-1,3-dioxolanes: Solvent Interaction Products in Some Commercial Beef Flavorings

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By use of a modified Likens and Nickerson extraction procedure followed by low-temperature/high-vacuum distillation, representative samples of aroma volatiles were obtained from some commercial beef flavorings. Prefractionation of concentrates by preparative gas chromatography facilitated subsequent analysis by combined gas chromatography/mass spectrometry. One fraction provided a number of similar mass spectra exhibiting intense characteristic peaks at m/e 87. It was considered that these could be 2-substituted-4-methyl-1,3-dioxolanes, but dioxolanes have not been reported as volatile components of meat. Selected 1,3-dioxolanes were synthesized and comparison of their mass spectra and GC retention times confirmed the identities of the unknown components of the simulated meat flavor isolate. Their origin may be explained by interaction of propane-1,2-diol, a commonly used solvent for commercial flavors, with carbonyl aroma components—interactions which can modify the flavor characteristics of the product.

Results of recent work undertaken at our laboratory involving the chemical and sensory analysis of cooked beef aroma have been reported (MacLeod and Coppock, 1976, 1977, 1978). As part of this continuing program, some commercial beef flavorings have been analyzed; these were mainly based on natural materials of plant or animal origin. Aroma extraction and concentration was achieved using previously reported techniques, optimized for cooked beef aroma isolation (MacLeod and Coppock, 1976), and the extracts obtained were analyzed by routine gas chromatography (GC). However, due to the complexity of the gas chromatograms, the total isolate was then fractionated by preparative GC into three fractions. The first of these, comprising the more volatile components, provided some initially unexpected results and these are the subject of this paper.

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EXPERIMENTAL SECTION

Sample Preparation. A concentrated total isolate of the aroma components of the commercial, simulated beef flavor was prepared as follows, based on the previously reported method for genuine cooked beef aroma isolation

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