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MELTABILITY AND RHEOLOGY OF MODEL PROCESS CHEESE

CONTAINING ACID AND RENNET CASEIN

by

Paul Alexander Savello

DEPARTMENT OF NUTRITION
& FOOD SCIENCES
Utah State University
750 North 1200 East
Logan Utah 84322-8700

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

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Paul A. Savello

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ABSTRACT

Meltability and Rheology of Model Process Cheese
Containing Acid and Rennet Casein

by

Paul A. Savello, Doctor of Philosophy

Utah State University, 1983

Major Professor: Dr. C. Anthon Ernstrom
Department: Nutrition & Food Sciences

Process cheese models were prepared by blending acid or rennet casein, milk fat, sodium chloride, 2.5% emulsifying salt and water and heating to 80 C. Acid casein cheese models were subjected to sodium hydroxide conditioning at 65 C in the cooker. Model process cheeses were acidified with lactic acid and treated by addition of undenatured and heat-denatured whey protein, four different emulsifying salts and sodium oxalate.

Meltability and toughness of the model cheese increased to a maximum with increased sodium hydroxide conditioning of acid casein to pH 7.20. These same properties decreased with addition of undenatured and heat-denatured whey protein to both casein cheese models. Loss of emulsion occurred

during the meltability test of rennet casein cheese models with 3.0 and 4.5% added whey protein.

Emulsifying salts affected the models differently. Disodium phosphate and tetrasodium pyrophosphate in rennet casein models eliminated the melting property. These same salts in acid casein models produced excellent meltability. Trisodium citrate produced cheeses with good meltability in both acid and rennet casein cheese models. Acid casein cheese models prepared with sodium aluminum phosphate had fair meltability and were very tender (no rupture upon compression). Chelation of calcium by sodium oxalate in rennet casein cheese emulsified with disodium phosphate or tetrasodium pyrophosphate improved meltability with a corresponding increase in toughness.

Scanning electron micrographs of model process cheeses indicated a direct relationship between extent of emulsification and poor meltability of rennet and pH conditioned acid casein model cheeses. Acid casein model cheeses prepared with different emulsifying salts did not exhibit this same relationship. Addition of whey protein concentrate to rennet casein model cheese produced fibrous structures around the fat globules. No structural abnormalities were noted in the acid casein cheeses prepared with whey protein concentrate.

INTRODUCTION

The utilization of ultrafiltered skim or whole milk retentate in the manufacture of cheese was first proposed by Maubois and Mocquot (24). Process cheese has been manufactured in which ultrafiltered milk retentate was used as a partial substitute for natural cheese. Process cheeses made with more than 40% plain retentate or 60% enzyme-treated retentate solids produced a cheese with long-grained texture and decreased meltability (43).

Ernstrom et al. (9) prepared a cheese base (38% moisture) by vacuum evaporating a cultured, ultrafiltered whole milk retentate. The cheese base was used as a substitute for 80% of natural cheese in process cheese and process cheese food production. The flavor of both products was good; the texture of the process cheese food was good while that of the process cheese was stiff.

Presently, process cheese made from ultrafiltered-prepared cheese base has minimal or no meltability as well as a brittle and tough texture. The meltability defect was not corrected by extensive proteolysis (up to 65% soluble nitrogen in 12% TCA) or by increasing the moisture level of the process cheese (preliminary results from this laboratory).

Cheese base composition is similar to natural cheese for processing with three exceptions: 1) milk serum proteins

and 2) the glycomacropeptide portion of k-casein are retained in the cheese base whereas these constituents are normally lost in the whey during natural cheese manufacture; 3) the calcium content of cheese base is higher than that of natural cheese (0.88 and 0.70%, respectively) (9,58).

Lonergan (26) reported that the casein micelles do not change in structure nor calcium and phosphorus composition during ultrafiltration and diafiltration. Thus, the textural changes of cheese prepared with ultrafiltered milk retentate are not due to casein micellar changes.

The functionalities of whole casein (i.e. isoelectric casein), rennet casein, and whey proteins in process cheese have not been extensively reported. Calcium content and protein structure differences between the two casein types permit their use to investigate the role of these constituents in causing the meltability defect noted in process cheese base.

The purpose of the present research is to design and test a model process cheese containing the constituents present in cheese base in order to identify the cause(s) of the meltability defect encountered when process cheese is prepared from cheese base.

LITERATURE REVIEW

The historical perspectives of growth and development of the process cheese industry have been presented by Price and Bush (35,36). Presently the annual natural cheese production in the United States is 2.8 billion pounds of which 60% (1.7 billion pounds) is used for processing (51). Clearly, the economic importance of the process cheese industry cannot be over-emphasized.

The new technologies of membrane ultrafiltration (UF) and separation can provide for increased yield of cheese products prepared from UF concentrated milk (9,25). A yield increase of 18% as reported by Ernstrom et al. (9) translates to an annual increase of \$400 million in process cheese value.

Functionality of Casein in Process Cheese

The use and functionality of rennet and acid casein in process cheese have not been widely reported. Rennet casein is used to prepare imitation process cheese products because it provides the desired characteristics of texture, meltability, and nutritive value of the final product (50). The protein structure and calcium content of rennet casein closely resembles natural cheese permitting its use as the

casein source in cheese products. Acid casein, however, contains no bound calcium (60) and has a different protein structure reflecting its significantly different method of manufacture.

A process for producing an imitation cheese food was patented in 1980 (28). A calcium caseinate solution was prepared by reacting an acid-precipitated casein suspension with a basic calcium salt at pH 7. Acid was added to decrease the pH to 5.9-6.9 followed by enzyme-coagulation of the caseinate at 80-110 F. The calcium caseinate curd was mixed with an oil, sodium chloride and an emulsifying salt. The blend was cooked at 140 F with additional acid to pH 5. Although the original suspension was acid-precipitated casein, the curd used in preparing the final product was a rennet casein. The functional properties of this curd would more closely relate to a rennet casein.

Lazaridis and Rosenau (20) reported successful functionality of direct acid casein curd used in a process cheese-like product. Wet acid casein curd was pressed, ground, heated and mixed with other formulation ingredients at 80 C for 5 min prior to addition of 6 N NaOH (to pH 8.0) as a "protein solubilization" step. Following emulsification the pH of the blend was lowered to 5.5 with 5 N HCl. The product was labeled as a "non-fermented, non-renneted, processed cheese". The type of emulsifying salt used in the process affected the meltability: disodium

and trisodium phosphate produced excellent melting products; cheese made with trisodium citrate melted slightly less than the phosphate salts.

The use of acid casein curd and cream as an extender in process cheese has been reported (39). Direct acid casein curd (50% moisture) was prepared from skim milk by acidification, heating, and centrifuging. The curd was mixed with plastic cream, salt, disodium phosphate, water, and varying levels of aged cheese. The blend was processed with direct steam injection. The product with 100% acid curd (i.e. no aged cheese added) was reported to have good body and texture.

Functionality of Emulsifying Salts in Process Cheese

The mode of action of effective emulsifying salts in process cheese is not clearly understood. Numerous theories have been proposed since the mid-thirties (40). It is generally accepted that during process cheese manufacture the emulsifying salt chelates some calcium that is bound to the para-casein (4,11,29,31,40,48) causing a disaggregation of casein with the subsequent formation of the more soluble sodium caseinate through ion exchange. The results of Nakajima et al. (31) showed that an orthophosphate salt mixed with isolated casein micelles reacted with colloidal calcium but did not affect the calcium bound to casein.

Disodium phosphate (DSP) has been characterized as an emulsifying salt with poor calcium binding capacity (4).

Tetrasodium pyrophosphate (TSPP) has also been shown to have very low Ca sequestering ability at pH 5-6 in dilute salt solutions (4,15). Leviton (23) showed that pyrophosphates associated with the caseinate-phosphate complex. He postulated that this resulted in cross-linkages and restructuring of the micelles. Morr (29) contrasted this with the finding that TSPP-treated skim milk caused protein dissociation and disaggregation by an alteration of the caseinate-phosphate micelles. Nakajima et al. (31) theorized that TSPP was adsorbed by casein when mixed with colloidal phosphate free casein.

Quantitation of calcium-chelating capabilities of various salts has been reported (15,54). These determinations have been performed using pure salt solutions. The sequestering potentials of the emulsifier salts may not be the same when included in a complex material such as process cheese. The calcium-protein complex could provide different physico-chemical parameters resulting in different chelating capabilities by the emulsifying salts.

Templeton and Sommer (47) reported that process cheese prepared with meta- or pyrophosphate emulsifying salts did not melt well; citrate salts (sodium and potassium) effectively produced a process cheese with excellent meltability.

Albonico and Gianani (1) investigated the calcium complexing action of three emulsifying salts. Orthophosphates and polyphosphate were similar in this action with citrate having a higher calcium complexing potential.

The effect of adding emulsifying salts to isolated skim milk casein micelles was investigated by Nakajima et al. (31). Distribution patterns of calcium and phosphorus after reacting the salt with the micelles indicated that the various phosphates and citrates tested acted differently. Orthophosphate reacted with colloidal calcium but did not affect calcium bound to casein; citrate chelated casein-bound calcium providing a greater degree of potential emulsifying action.

Rayan et al. (38) reported a significant difference between various emulsifying salts on meltability of process cheese. Trisodium citrate and sodium aluminum phosphate (5,18,40,46) produced process cheese with better cheese flow than cheeses prepared with disodium or trisodium phosphate.

Vujicic et al. (56) reported that casein acts as a multivalent cation which is effective in replacing sodium and hydrogen ions from polyphosphates and citrate. The research indicated that the presence of casein in polyphosphate and citrate solutions increased the dissociation of sodium from the salts. Upon addition of calcium to the system (as a soluble calcium salt) the release of sodium from the emulsifying salts was complete.

Functionality of Whey Protein
Concentrates

The functional properties of whey protein in food systems have centered around gelation, whippability, and foaming capabilities. Schmidt and Illingworth reported the close similarity between a heat-induced whey protein gel to the gelation properties of egg white protein (41). McDonough et al. (27) showed that a 10% whey protein concentrate solution (50% whey protein) formed a firm gel when heated to 85 C. The gelation phenomenon was interpreted as the formation of a three-dimensional structure that could entrap water, thereby producing a gel resembling a heat-induced egg white gel.

Morr (30) indicated that maximum gel strength of a 10% whey protein concentrate solution occurred in the presence of 11 mM calcium ions and heated to 100 C for 15 min. A protein-protein interaction due to involvement of calcium ions and ionic bonding was suggested. The gelation properties of a whey protein concentrate solution depended on numerous parameters: ionic strength of the solution, heating conditions to produce the gel, and concentration of divalent cations (such as Ca^{++}) (30).

Fox and Mulvihill (10) reported that a minimum whey protein concentration was necessary for gelation to occur. The gelling time was reduced as the protein concentration

increased above the minimum. Gelling time was also reduced as the temperature increased; however, after heating above 90 C the gel formation occurs only upon cooling of the treated solution.

It has been hypothesized that calcium interacts with specific groups of whey protein. This causes a reduced net negative charge of the protein to zero, thereby causing isoelectric precipitation of the protein (59).

Heat coagulated whey protein incorporated into a process cheese formulation did not blend properly, giving the product a grainy texture (3). However, when whey protein was prepared by adding 0.5% calcium chloride to sweet whey, proper pH adjustment made with HCl, heating, and filtering, the whey protein incorporated satisfactorily into the process cheese (50-55% moisture) with no textural defects. No meltability results were reported for the product.

Whey protein concentrate (21% solids, 16.6% protein) was incorporated into a process cheese formulation at 10% (w/w) level. No significant change in textural qualities were noted (16).

Cheese Rheology

Objective measurements of cheese textural characteristics have included rheologic studies of natural

and process cheeses. Standardization of procedures in making such measurements does not exist making it difficult to compare results of different investigations.

Firmness measurements of process Tilsit cheese showed that the emulsifying salts trisodium citrate and disodium phosphate produced the softest cheese while polyphosphate produced the firmest cheese (44).

Gouda cheese (7) and Leicester cheese (55) firmness tests were determined with an Instron Universal testing machine. Friction effects between the cheese sample (cut in shape of a cylinder) and the instrument compression plates caused different compression results: when mineral oil was spread between the cheese and plate surfaces a concave deformation of the cheese cylinder occurred; with emery paper placed between the surfaces the resulting deformation was convex and barrel-shaped. It was noted that these friction effects were important in determining firmness and hardness characteristics of the cheese samples.

Rayan et al. (38) reported that use of tetrasodium pyrophosphate as an emulsifying salt produced a very firm process cheddar cheese with very little meltability. Sodium aluminum phosphate in the formulation produced a very soft process cheese that exhibited good cheese flow.

Harvey et al. (12) reported a positive correlation between meltability and cohesiveness of process cheddar cheese. Other textural characteristics (including hardness,

springiness, gumminess, chewiness, fracturability, and adhesiveness) were not closely related to cheese meltability.

Microstructure of Process Cheese

Scanning electron microscopy has recently become an important aid in analyzing and correlating the physical properties of process cheese (e.g. meltability and firmness) to the cheese structure and degree of emulsification. Kimura et al. (19) and Taneya et al. (45) used scanning electron micrographs of process cheese to indicate that hard type cheese (prepared with pyrophosphate as an emulsifying agent) had sub-micelle structures linked together in string-like fashion. Soft process cheese prepared with a citrate-polyphosphate blend did not contain such structures.

Rayan et al. (38) reported that the extent of emulsification (evidenced by the fineness of fat globules) was related to process cheese firmness and poor meltability. The least meltable cheese was prepared with TSPP as the emulsifying agent. This cheese exhibited the highest and fastest degree of emulsification. Cheeses prepared with sodium aluminum phosphate or citrate had a less complete emulsification and, correspondingly, displayed a good meltability characteristic.

Heertje et al. (13) presented electron micrographs of process cheese that showed strand-like material in clearer

detail than previously reported (19,45). The dimensions of the material were 10 nm diameter and 300 nm in length. These strand dimensions are smaller than those observed in normal cheese. The authors interpreted the cause of these strands differently than other investigators: rather than being casein sub-micelles they favored a molecular association mechanism similar to heat-induced gelation of proteins such as ovalbumin, insulin, and lysozyme. The structures were formed by protein molecule unfolding followed by non-random aggregation in a network structure.

The effects of emulsifying salt concentration, cooking temperature, and product pH on the microstructure of process cheese were investigated by Lee et al. (21). Increasing the cooking temperature (to 140 C) and polyphosphate emulsifying salt concentration (to 4%) caused progressive dispersion of the casein micelles in the cheese. There was an increase in firmness (penetrometer measurements) of the cheeses as the salt concentration and cooking temperature increased. Cheeses prepared with pH values of 5.4 and 6.6 did not indicate differences by scanning electron microscopy.

MATERIALS & METHODS

Model System Process Cheese IngredientsCasein

Rennet casein (Alacase 771) and lactic acid casein (Alacase 710) were purchased in 25 kg bags from New Zealand Milk Products, Inc. (Petaluma, CA 94952). Both caseins were obtained in 30 mesh size.

Butter

Commercial butter manufactured by Cache Valley Dairy Association (Logan, UT 84321) was purchased as 454 g blocks in a local supermarket.

Whey Protein

Modified whey protein (WP) powder (approximately 36% whey protein) was obtained in 25 kg bags from Ward's Cheese Co. (Richfield, ID 83349).

Lactic Acid

Lactic acid (grade DL-III, approximately 85% syrup) was purchased from Sigma Chemical Co. (St. Louis, MO 63178).

Emulsifying Salts

Emulsifying salts were among those legally permissible for use in pasteurized process cheese.

- 1) Trisodium citrate (CIT): Miles Laboratories, Inc., Elkhart, IN 46515 ;
- 2) Disodium orthophosphate (DSP): Stauffer Chemical Co., Westport, CT 06880;
- 3) Sodium aluminum phosphate (SALP): Stauffer Chemical Co., Westport, CT 06880;
- 4) Tetrasodium pyrophosphate (TSPP): Stauffer Chemical Co., Westport, CT 06880.

Chemicals

All chemicals used in preparation of process cheese and subsequent chemical analyses were reagent grade.

Preparation of Freeze-Dried Whey

Protein Powder

Undenatured Whey Protein Concentrate

Seventy five kilograms of a 15% modified whey protein solution in deionized water was prepared. The solution was ultrafiltered (batch-wise) at 25 C in an Abcor HFK-130 single-stage, spiral-wound, polysulfone membrane. The ultrafiltration process was performed with 420 kPa (60 psi) inlet pressure and 280 kPa (40 psi) outlet pressure on the membrane.

Diafiltration of the solution was performed to remove lactose and salts. The diafiltration step was effected with

225 kg of deionized water added in 25 kg batches to the whey protein solution to maintain the solution at maximum volume in the feed tank.

Following diafiltration, the solution was concentrated to one-fourth the original solution volume. The concentrated solution was frozen in stainless steel trays (29 x 42.5 x 4 cm) and freeze-dried in a Dura Dry Freeze Drier (FTS Systems, Inc., Stone Ridge, NY 12484). The freeze-dried undenatured whey protein concentrate (UWPC) was stored in plastic bags.

Denatured Whey Protein Concentrate

Two hundred fifty kilograms of 2.8% modified whey protein solution (1% whey protein) was prepared in deionized water. The solution (pH 6.60) was heated to 85 C and held at that temperature for one hour (58). Following the heat treatment, the solution was cooled to 50 C, ultrafiltered, diafiltered with 625 kg of deionized water, and concentrated to one-tenth the original solution volume. The denatured whey protein concentrate (DWPC) solution was frozen, freeze-dried, and stored in plastic bags as described above.

Preparation of Model Process

Cheese Samples

All model process cheese samples were prepared in duplicate as 2 kg batches in a specially built

scraped-surface, batch cooker with three kilogram capacity (Figure 1). The scraper blades of the cooker were maintained at 120 rpm throughout the cooking procedure. Indirect heating was from a steam jacket surrounding the bowl.

Rennet Casein Process
Cheese Model

The basic rennet casein model process cheese formulation included:

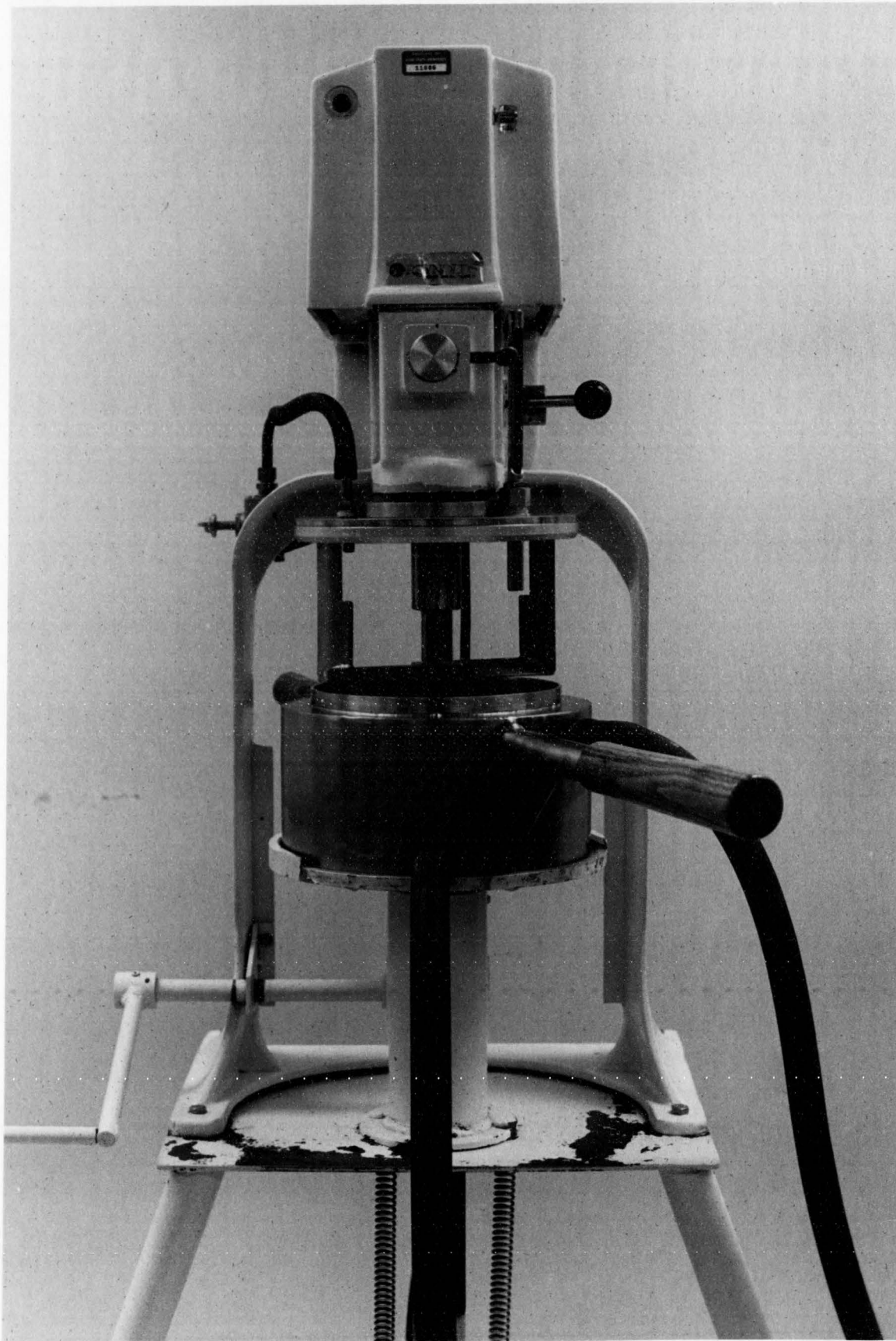
- 1) 770 g butter
- 2) 483 g rennet casein
- 3) 16 g sodium chloride
- 4) 50 g emulsifying salt
- 5) 676 g water (deionized).

This formulation yielded process cheese with 39-40% moisture, 20-22% protein, 52-54% fat-in-dry matter, 4.5% salt-in-moisture, and 2.5% emulsifying salt.

The formulation was prepared in the following manner:

- 1) Butter was melted in the cooker at 50 C;
- 2) Dry ingredients were added to and blended with the butter;
- 3) The mix was heated to 65.6-68.3 C;
- 4) Lactic acid in the required amount of water was added to lower the pH of the resulting process cheese to 5.65-5.75;
- 5) The blend was maintained at 65.6-68.3 C and stirred

Figure 1. Custom batch cooker of three kilogram capacity.



for four minutes;

6) The blend was heated to 82.3 C and held at this final cook temperature for 1 min.

Acid Casein Process
Cheese Model

The basic acid casein process cheese formulation included:

- 1) 770 g butter
- 2) 486 g acid casein
- 3) 16 g sodium chloride
- 4) 50 g emulsifying salt
- 5) 676 g water (deionized)

This acid casein model yielded process cheese with 39-40% moisture, 20-22% protein, 52-54% fat-in-dry-matter, 4.5% salt-in-moisture, and 2.5% emulsifying salt.

The formulation was prepared in the following manner:

- 1) Butter was melted in the cooker at 50 C;
- 2) Dry ingredients were added to the melted butter;
- 3) A measured amount of 5 N NaOH in 80% of the required amount of water was added to the cooker;
- 4) The mix was heated to 65.6-68.3 C and blended at this temperature for four minutes;
- 5) Lactic acid in the remaining 20% of the required water was added to lower the pH of the resulting process cheese to 5.65-5.75;
- 6) The blend was heated to 83.3 C and held at this final cook temperature for 1 min.

Rennet Casein Model Process
Cheese with Different
Emulsifying Salts

Four different emulsifying salts were used in the rennet casein model. The preparation of process cheese samples using different emulsifying salts was identical to that described above with the exception of the samples made with TSPP. In the preparation of this sample, 65% of the required lactic acid was mixed with water (step 4 of the basic rennet casein model system) and added to the cooker. The remaining lactic acid was added to the cooker when the temperature of the blend reached 75 C. This salt lost its emulsifying capacity if all the lactic acid was added to the blend. The fat separated at 65.6-68.3 C; the protein mass became sticky and clung to the cooker blades, decreasing both heat transfer and the internal mixing needed to reincorporate the fat into the emulsion. The process cheese blend was cooked and packaged as previously described.

Acid Casein Model Process
Cheese with Different
Emulsifying Salts

Four different emulsifying salts were used in the acid casein model. Process cheese samples were prepared using 65 mL of 5 N NaOH in 80% of the required amount of water (step 3 of the basic acid casein model system). The amount of lactic acid in the remaining 20% of the required

water was adjusted to lower the pH of the process cheese sample to 5.65-5.75.

Rennet Casein Model Process
Cheese with Undenatured
Whey Protein

Freeze-dried UWPC was added to the rennet casein model to yield 1.5, 3.0, and 4.5% whey protein in the final product. An equivalent amount of rennet casein was withheld from the formulation in order to maintain constant protein and total solids in the sample. All samples were prepared with CIT as the emulsifying salt.

The rennet casein model process cheese with UWPC was prepared as follows:

- 1) Butter was melted in the cooker at 50 C;
- 2) Rennet casein, sodium chloride, and emulsifying salt were added to the cooker;
- 3) The mix was heated to 65.6-68.3 C;
- 4) Lactic acid in the required amount of water was added to lower the pH of the resulting process cheese to 5.65-5.75;
- 5) The mix was blended at 65.6-68.3 C for four minutes;
- 6) The mix was heated to 73.9 C;
- 7) UWPC was added to the cooker;
- 8) The blend was heated to 83.3 C and held at this temperature for 1 min.

Acid Casein Model Process
Cheese with Undenatured
Whey Protein

Freeze-dried UWPC was added to the acid casein model to yield 1.5, 3.0, and 4.5% whey protein in the final product. An equivalent amount of acid casein was withheld from the formulation to maintain constant protein and total solids in the samples. All samples were prepared with CIT as the emulsifying salt.

The acid casein model process cheese with UWPC was prepared as follows:

- 1) Butter was melted in the cooker at 50 C;
- 2) Acid casein, sodium chloride, and emulsifying salt were added to the cooker;
- 3) 65 mL of 5 N NaOH in 80% of the required amount of water was added to the cooker;
- 4) The mix was heated to 65.6-68.3 C and blended at this temperature for four minutes;
- 5) Lactic acid in the remaining 20% of the required water was added to lower the pH of the blend to 5.65-5.75;
- 6) The blend was heated to 73.9 C;
- 7) UWPC was added to the cooker;
- 8) The blend was heated to 83.3 C and held at this final cook temperature for 1 min.

Rennet Casein Model Process
Cheese with Denatured
Whey Protein

Freeze-dried DWPC was added to the rennet casein model to yield 1.5, 3.0, and 4.5% whey protein in the final product. An equivalent amount of rennet casein was withheld from the formulation to maintain constant protein and total solids in the samples. All samples were prepared with CIT as the emulsifying salt.

Preparation of the rennet casein model system process cheese with DWPC was identical to the preparation of model system process cheese with native whey protein powder.

Acid Casein Model Process
Cheese with Denatured
Whey Protein

Freeze-dried DWPC was added to the acid casein model to yield 1.5, 3.0, and 4.5% whey protein in the final product. An equivalent amount of rennet casein was withheld from the formulation to maintain constant protein and total solids in the samples. All samples were prepared with CIT as the emulsifying salt.

The preparation of the acid casein model process cheese with DWPC was identical to the preparation of model process cheese with UWPC.

Rennet Casein Model Process Cheese
with Disodium Oxalate as a
Calcium Binding Agent

Disodium oxalate (36.9 g) was added to the basic rennet casein model to act as a calcium binding agent. Quantities of all dry ingredients in the formulation remained the same; an additional 15 mL of deionized water was added to maintain constant total solids in the samples. DSP or TSPP was used as emulsifying salt in all samples.

Packaging and Storage

All model process cheese samples were packaged in 0.454 kg round plastic containers and stored at 2 C until use.

Chemical Analyses

Chemical analyses were performed on raw ingredients used in the model systems (caseins, whey protein concentrates and butter) and on the final process cheese samples.

Moisture

Cheese and ingredient moisture determinations were made in duplicate by heating an accurately weighed sample in an oven at 110 C for 16 hours (37). Process cheese samples were finely grated prior to moisture determinations. The

weight loss due to heating was considered as water loss from the sample.

Protein

Protein determinations on casein, WPC and cheese were made in duplicate by semi-micro Kjeldahl procedure for nitrogen (14). Protein content was calculated by multiplying the nitrogen content of the sample by the factor 6.38.

Fat

Fat in the process cheese was determined in duplicate by a modified Babcock method described by Van Slyke and Price (53). Fat in the butter was determined in duplicate by the Mojonnier test (32).

Soluble Protein at pH 4.40

Soluble protein in the process cheese samples was determined in duplicate according to the method of Vakaleris and Price (52). An accurately weighed 15.000 g of cheese was placed in a 32 x 200 mm test tube. Forty milliliters of 0.5 N trisodium citrate dihydrate solution (at 60 C) was added. The cheese was blended in the citrate solution. The cheese-citrate blend was transferred quantitatively to a 200 mL volumetric flask. The solution was brought to volume with deionized water. One hundred milliliters of the

cheese-citrate blend was accurately measured and placed in a 250 mL Erlenmeyer flask. An accurately measured volume of 1.47 N HCl was added to lower the pH of the blend to 4.40 ± 0.05 . The acidified blend was filtered through Whatman No. 42 filter paper. Ten milliliters of the filtrate was digested and the nitrogen content determined by a semi-micro Kjeldahl procedure (14). The protein content was calculated by multiplying the nitrogen content of the sample by 6.38.

pH

Eight grams of cheese was blended in 15 mL of deionized, glass-distilled water. The pH of the slurry was measured with an Orion pH/millivolt meter 811 and a single reference combination glass electrode (Orion Research Model 91-02, Orion Research, Inc., Cambridge, MA).

Calcium Determination

Calcium content of rennet and acid casein was determined in duplicate by atomic absorption spectrophotometry (2). An accurately weighed 2.500 g sample of rennet and acid casein was ashed in a furnace at 550 C. The ash residue was dissolved in 5 mL of 6 N HCl and brought to 25 mL volume with 1000 ppm lanthanum oxide solution. The sample was diluted to bring the calcium concentration into the linear range of the spectrophotometer for calcium determination.

Meltability

The model process cheese samples were tested for meltability in triplicate by using a modified meltability test according to Olson and Price (34). A cheese plug weighing 15.0 ± 0.1 g and measuring 30 mm diameter and approximately 22 mm long was placed at one end of a pyrex glass tube (30 mm I.D. and 250 mm long). This end of the glass tube was closed with a solid rubber stopper. The opposite end of the tube was closed with a one-hole (3 mm) rubber stopper. A reference line indicating the leading edge of the cheese plug was drawn on the outside of the glass tube.

The melting tubes were placed on a stainless steel rack and incubated at 30 C for 120 min. During this incubation period the melting tubes were placed on the rack at a 45 degree angle with the tube end containing the cheese plugs at the bottom. Following incubation the melting tubes and rack were placed in a horizontal position in an oven at 110 C for 50 min. The flow of melted cheese within the tubes was halted upon removal from the oven by slightly tilting the rack from horizontal. The distance of flow from the reference line to the leading edge of the melted cheese was measured in millimeters and recorded as "cheese flow".

Rheology Measurements

Rheology measurements of the cheese samples were made on an MTS Tensile Testing Machine Type T5002 (J.J. Lloyd

Instruments, Limited, Warsah, Southhampton, England). A two channel X-Y plotter was interfaced with the testing machine. A 500 Newton load cell was used for all rheology measurements. Cheese samples were stored at 15.5 C for 48 h prior to making all measurements.

Firmness

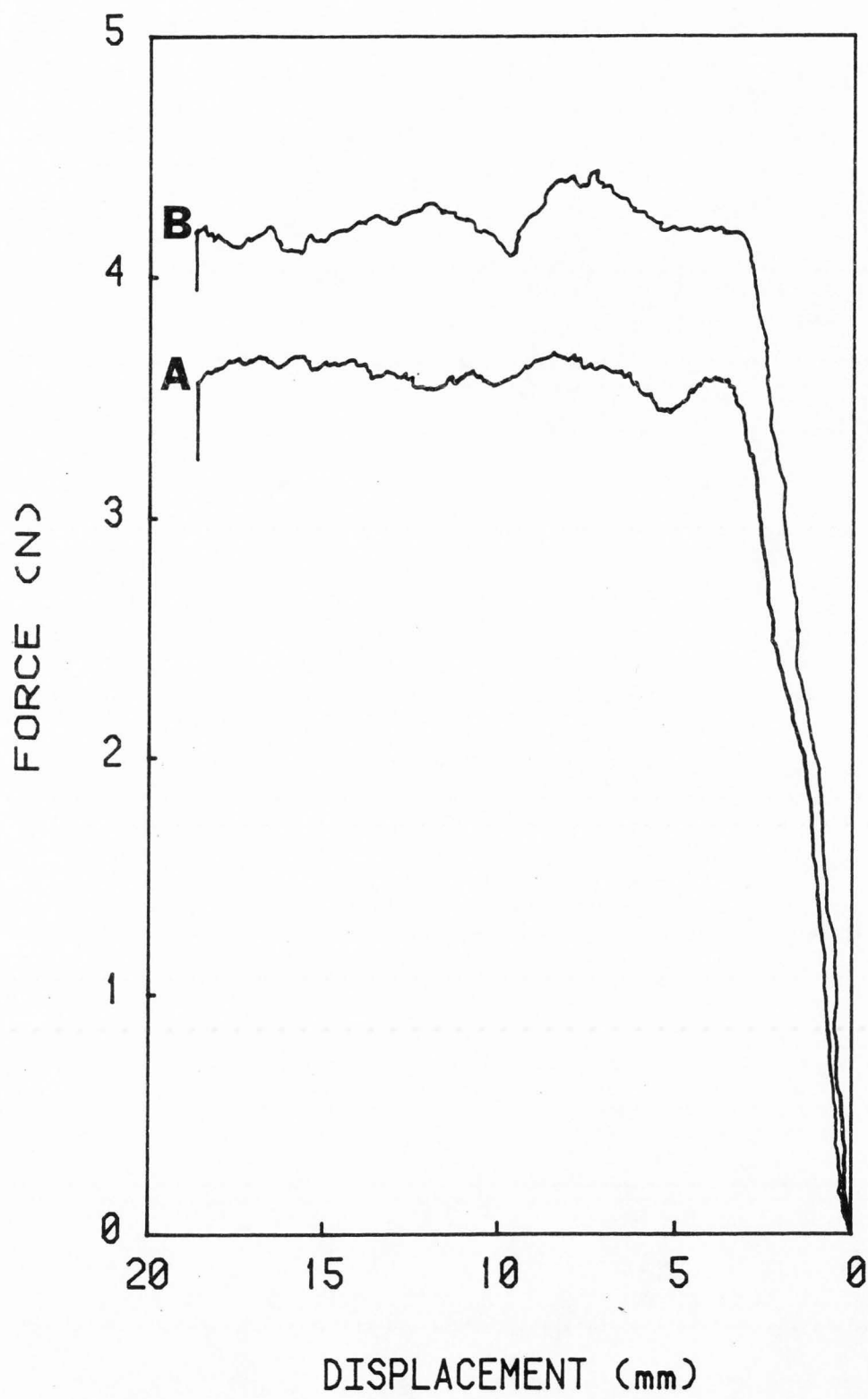
The testing machine was operated at a crosshead speed of 50 mm/min and paper/crosshead ratio of 10/1. The sensitivity setting for cheese samples with added whey proteins was 0.04; for all other samples the sensitivity setting was 0.01.

Cheese cylinders measuring 19 mm in diameter and 20 mm high were cut according to Rayan et al. (38). Firmness was measured according to the method of Emmons et al. (8) and as modified by Rayan et al. (38). The wire passed through 90% of the original sample height.

Figure 2 is a firmness plot (force vs displacement) for two cheese samples. Sample A is less firm than sample B. Approximately 2.5 mm displacement through a cheese cylinder was required to reach a force level that remained relatively constant throughout the firmness measurement.

Force measurements at 7 and 14 mm displacement were made for each of the triplicate sample plots. The average of the six firmness force values for each cheese sample was calculated.

Figure 2. Firmness plot (typical) of model process cheeses.



Toughness

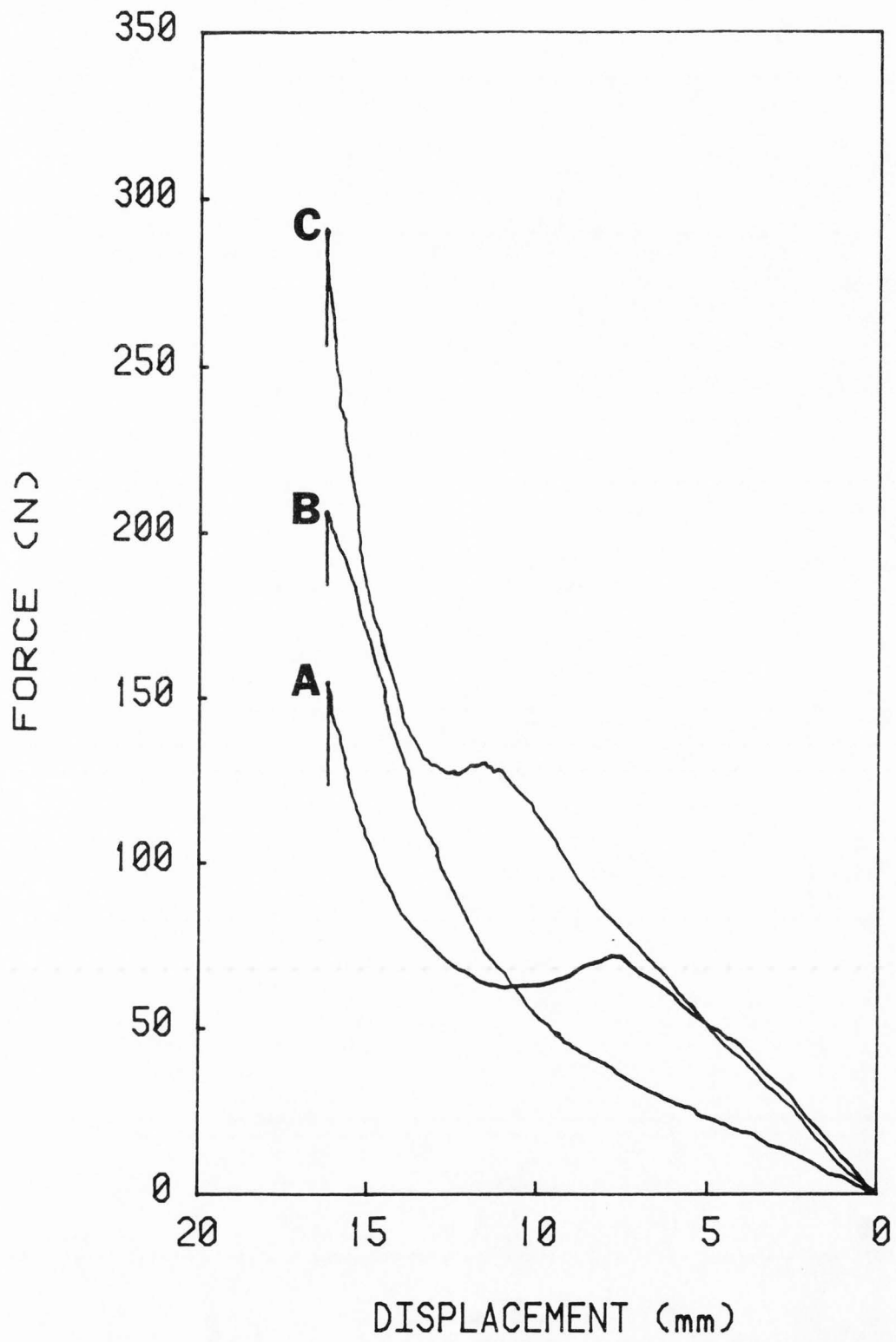
The testing machine was operated with a crosshead speed of 30 mm/min and a paper/crosshead ratio of 5/1. The sensitivity setting was 1.0 for all toughness tests. Pieces of waxed weighing paper were placed between the cheese cylinder and compression plates to reduce friction interference (7).

Toughness was determined by compressing a cheese cylinder (19 mm diameter x 20 mm height) between parallel plates to 20% of the original sample height (7). Toughness was calculated by measuring the area under the force-displacement curve to the right of a normal line drawn to the abscissa from the inflection (or yield) point of the curve (22,57). The inflection (or yield) point of the curve indicates the initial rupture of the cheese sample.

Figure 3 depicts three force-displacement curves of representative cheese samples. Sample A had an inflection point at 73.5 N and 7.5 mm displacement. Sample B did not have an inflection point indicating that the sample did not rupture during the compression. Sample C had an inflection point at 121.6 N and 13.9 mm displacement indicating a toughness level higher than Sample A.

The area under the curve was measured using a Tektronix 4052 microcomputer interfaced with a Tektronix Interactive Digital Plotter 4662. A sight glass with crosshairs was placed in the plotter's pen tracking guide. The plotter's

Figure 3. Toughness plot (typical) of
model process cheeses.



manual tracking guide control was used to track the force-displacement line. A total of 125 points at 0.3 sec interval was recorded in the computer's memory. The area under the curve was calculated according to the program in Appendix A.

Scanning Electron Microscopy of Model Process Cheese

Process cheese specimens were prepared for SEM by fixing a 5 mm cheese cube in 1.4% glutaraldehyde solution, dehydrating in a graded alcohol series, defatting in chloroform, and critical-point drying from carbon dioxide. Dry specimens were fractured and the fragments mounted on SEM stubs, coated with carbon and gold by vacuum evaporation. Specimens were examined under a Cambridge Stereoscan electron microscope operated at 20 kv (17).

Statistical Analysis

Meltability Data

A randomized block experimental design was used in all meltability tests. Cheese samples were randomly placed in the melting rack slots. Randomization of each set of process cheese samples was carried out for each of the triplicate melt tests performed.

Analysis of variance was performed to determine the significance of meltability among the cheese samples.

Where significance occurred among samples a Newman-Keul multiple range test (6) was used to determine significance between sample pairs.

Rheology Data

Completely randomized firmness and toughness measurements were taken of all samples. Analysis of variance was performed to determine the significance of firmness and toughness among the following blocks of cheese samples:

1. rennet and acid casein process cheeses;
2. rennet and acid casein process cheese prepared with different emulsifying salts;
3. rennet and acid casein process cheese prepared with UWP or DWP.

Analysis of variance of block 3 above included the testing of orthogonal contrasts (equal to treatment degrees of freedom) for significance. The contrasts included:

1. WP present vs. WP absent;
2. Acid casein vs. rennet casein (WP absent);
3. Acid casein vs. rennet casein (WP present);
4. Undenatured WP vs. Denatured WP;
5. (Casein type) x (WP Type) interaction;
6. Linear effect of undenatured WP (acid casein);
7. Quadratic effect of undenatured WP (acid casein);
8. Linear effect of denatured WP (acid casein);

9. Quadratic effect of denatured WP (acid casein);
10. Linear effect of undenatured WP (rennet casein);
11. Quadratic effect of undenatured WP (rennet casein);
12. Linear effect of denatured WP (rennet casein);
13. Quadratic effect of denatured WP (rennet casein);

Appendix B lists the treatments and orthogonal contrast coefficients used in determining the sums of squares for statistical analysis.

RESULTS

Model Process Cheese Ingredients

Composition of the model process cheese ingredients is presented in Table 1. The results are means of duplicate determinations.

Table 1. Analysis of process cheese ingredients.

Ingredient	Moisture (%)	Protein (Nx6.38) (%)	Ash (%)	Fat (%)	Calcium (mg/g)
Acid casein	10.5	90.4	0.48	*	0.00
Rennet casein	10.9	84.2	8.02	*	22.6
Whey Protein Concentrate (Undenatured)	7.35	72.8	3.11	*	6.17
Whey Protein Concentrate (Denatured)	6.85	72.9	*	*	*
Butter	17.5	*	*	80.1	*

* Not determined.

Sodium Hydroxide and Lactic Acid Addition
to Model Process Cheese Formulations

The volumes of 5 N NaOH and 80% lactic acid added to 2 kg batches of the process cheese formulations are recorded in Table 2. As the volume of NaOH increased in the conditioning of acid casein, the volume of added lactic acid increased to provide a uniform final pH of the product.

Table 3 records the volumes of 5 N NaOH and 80% lactic acid added to the formulations prepared with different emulsifying salts. Each salt had a unique buffering capacity in the formulation; the volume of added lactic acid varied accordingly to result in the desired final product pH. The acid casein formulation with SALP, DSP, and TSPP required more lactic acid than the corresponding rennet casein formulation; less lactic acid was required in the acid casein model when CIT was used as the emulsifying salt.

Cooking Conditions of Model Process Cheese

Tables 4 and 5 record the product composition and cooking conditions when undenatured or heat-denatured WPC, respectively, was added to the model cheese formulation. As the amount of WPC was increased in the formulations, the casein weight was decreased in order to maintain a constant protein content. The volume of lactic acid added to acid casein formulations, necessary to lower the pH of the final product to the desired level, was increased accordingly.

Table 2. Sodium hydroxide and lactic acid additions to 2 kg batches of rennet and acid casein model process cheese.

Casein Type	5 N NaOH (mL)	Conditioning ¹ pH	Water Added		80% Lactic Acid (mL)	Cook Time 66 to 82 C (min)
			with NaOH (mL)	with Acid (mL)		
Rennet	-	-	-	645	33	4.00
Acid	25	5.80	515	137	-	3.75
Acid	35	6.05	505	131	6	4.00
Acid	45	6.35	495	128	9.5	3.75
Acid	55	6.70	485	122	15	3.75
Acid	65	7.00	475	117	22	3.75
Acid	75	7.30	465	107	30	3.80

¹ pH measured after addition of 5 N NaOH and blending for four minutes at 66 C (prior to lactic acid addition).

Table 3. Sodium hydroxide and lactic acid additions to 2 kg batches of model process cheese prepared with different emulsifying salts.

Sample		Water Added					
Emulsifying Salt	Casein Type	5 N NaOH (mL)	Conditioning ¹ pH	with NaOH (mL)	with acid (mL)	80% Lactic Acid (mL)	Cook Time 66 to 82 C (min)
SALP	Acid	65	7.65	475	120	37	4.00
DSP	Acid	65	7.55	475	120	44	3.50
TSPP	Acid	65	7.75	475	120	46	3.60
CIT	Acid	65	6.95	475	120	22	3.60
SALP	Rennet	-	-	-	655	25	4.60
DSP	Rennet	-	-	-	650	30	4.60
TSPP	Rennet	-	-	-	650	36	5.30
CIT	Rennet	-	-	-	650	30	5.30

¹ pH measured after addition of 65 mL 5 N NaOH and emulsifying salt and blending for four minutes at 66 C (prior to lactic acid addition).

Table 4. Cooking conditions of model process cheese with added undenatured whey protein concentrate.

Sample						
Whey Protein (%)	Casein Type	UWPC (g)	Casein (g)	80 % Lactic Acid (mL)	Cook Time 66 to 74 C (min)	Cook Time 74 to 82 C (min)
0.0	Acid	0	486	22	-	4.20 ¹
1.5	Acid	41	445	22	1.50	2.75
3.0	Acid	83	404	25	1.60	3.00
4.5	Acid	124	363	30	1.40	3.00
0.0	Rennet	0	486	33	-	4.25 ¹
1.5	Rennet	41	445	33	1.50	3.30
3.0	Rennet	83	404	33	1.25	3.50
4.5	Rennet	124	363	33	1.00	3.00

¹ Cook time from 66 to 82 C.

Table 5. Cooking conditions of model process cheese with added denatured whey protein concentrate.

Sample						
Whey Protein (%)	Casein Type	DWPC (g)	Casein (g)	80% Lactic Acid (mL)	Cook Time 66 to 74 C (min)	Cook Time 74 to 82 C (min)
1.5	Acid	41	445	22	1.50	3.00
3.0	Acid	82	404	25	1.30	3.25
4.5	Acid	124	363	30	1.40	3.50
1.5	Rennet	41	445	33	1.10	3.40
3.0	Rennet	82	404	33	1.30	3.80
4.5	Rennet	124	363	33	1.00	3.60

During cooking, the cheese models prepared with added DWPC had a stiffer consistency than those prepared with the same concentration of UWPC. In both model process cheeses (acid and rennet casein), the samples became stiffer as WP content increased and attained a doughy consistency at the final cook temperature in samples with the highest levels of WP.

Composition of Model Process Cheese

Tables 6-9 record the composition of the model process cheeses. Moisture, protein, fat-in-dry-matter, and pH of all samples were within the desired ranges.

Soluble Protein in Model Process Cheese with Added Whey Protein Concentrate

Cooking of the process cheese models with added UWPC resulted in heat-denaturation of some whey protein (Table 10). The UWPC contained more than 91% soluble protein (pH 4.40) expressed as percent of total whey protein in the freeze-dried powder. After cooking the cheese formulations only one-half of the whey protein (from 38.6 to 50.4%) was soluble at pH 4.40. In contrast to the cheeses with added UWPC, samples containing DWPC did not decrease in pH 4.40 soluble protein during cooking. The heat treatment during the cooking of cheese containing DWPC did not reduce whey

Table 6. Composition¹ of rennet and acid casein model process cheese.

5 N NaOH (mL)	Casein Type	Moisture (%)	Protein (N x 6.38) (%)	Fat-in- Dry Matter (%)	pH
-	Rennet	39.7 ± 0.39	20.2 ± 0.31	53.3 ± 0.82	5.70
25	Acid	40.5 ± 0.18	21.6 ± 0.30	54.6 ± 0.58	5.69
35	Acid	40.5 ± 0.12	21.8 ± 0.44	55.1 ± 0.39	5.68
45	Acid	40.6 ± 0.16	21.2 ± 0.23	54.9 ± 0.78	5.74
55	Acid	39.5 ± 0.59	21.6 ± 1.04	52.5 ± 0.95	5.73
65	Acid	39.5 ± 0.83	21.7 ± 0.29	52.4 ± 0.61	5.67
75	Acid	38.8 ± 0.57	21.6 ± 0.56	52.1 ± 1.24	5.62

¹ Mean ± standard deviation of duplicate determinations of duplicate samples.

Table 7. Composition¹ of model process cheese prepared with different emulsifying salts.

Sample					
Emulsifying Salt	Casein Type	Moisture (%)	Protein (N x 6.38) (%)	Fat-in-Dry Matter (%)	pH
SALP	Acid	40.6 ± 0.13	20.1 ± 0.42	52.4 ± 0.70	5.75
DSP	Acid	40.4 ± 0.36	20.2 ± 0.62	53.5 ± 0.37	5.73
TSPP	Acid	39.8 ± 0.16	20.2 ± 0.82	52.6 ± 0.67	5.71
CIT	Acid	39.8 ± 0.18	20.6 ± 0.20	53.4 ± 0.93	5.67
SALP	Rennet	39.6 ± 0.72	18.9 ± 0.64	54.0 ± 0.35	5.75
DSP	Rennet	40.0 ± 0.16	18.9 ± 0.55	53.9 ± 0.78	5.74
TSPP	Rennet	38.9 ± 0.14	20.0 ± 1.51	53.4 ± 0.80	5.72
CIT	Rennet	38.9 ± 1.05	19.7 ± 0.42	52.4 ± 1.44	5.69

¹ Mean ± standard deviation of duplicate determinations of duplicate samples.

Table 8. Composition¹ of model process cheese with added undenatured whey protein concentrate.

Sample					
Whey Protein (%)	Casein Type	Moisture (%)	Protein (N x 6.38) (%)	Fat-in-Dry Matter (%)	pH
0.0	Acid	38.8 ± 0.55	22.0 ± 0.57	52.7 ± 0.40	5.58
1.5	Acid	38.9 ± 0.47	21.6 ± 0.61	53.2 ± 0.32	5.56
3.0	Acid	39.3 ± 0.17	21.1 ± 0.66	54.1 ± 0.95	5.64
4.5	Acid	39.1 ± 0.43	20.6 ± 0.32	53.9 ± 1.59	5.57
0.0	Rennet	39.3 ± 0.25	20.2 ± 0.37	54.5 ± 0.55	5.63
1.5	Rennet	38.8 ± 0.13	19.8 ± 0.47	54.5 ± 0.36	5.59
3.0	Rennet	38.5 ± 0.47	20.0 ± 0.34	55.1 ± 1.33	5.60
4.5	Rennet	38.5 ± 0.55	19.1 ± 1.19	55.7 ± 1.17	5.59

¹ Mean ± standard deviation of duplicate determinations of duplicate samples.

Table 9. Composition¹ of model processes cheese with added denatured whey protein concentrate.

Sample					
Whey Protein (%)	Casein Type	Moisture (%)	Protein (N x 6.38) (%)	Fat-in-Dry Matter (%)	pH
1.5	Acid	39.8 ± 0.46	20.8 ± 0.47	52.8 ± 0.63	5.66
3.0	Acid	38.6 ± 1.87	22.1 ± 1.14	53.1 ± 0.73	5.70
4.5	Acid	39.1 ± 0.43	20.2 ± 0.76	52.9 ± 1.11	5.63
1.5	Rennet	40.1 ± 0.16	19.8 ± 0.90	55.7 ± 0.36	5.60
3.0	Rennet	39.8 ± 0.22	19.6 ± 1.59	54.6 ± 0.62	5.60
4.5	Rennet	39.7 ± 0.16	19.5 ± 1.24	55.4 ± 0.77	5.61

¹ Mean ± standard deviation of duplicate determinations of duplicate samples.

Table 10. Soluble protein in process cheese with added whey protein concentrate.

Whey Protein (%)	Casein Type	Whey Protein Type	$\frac{\text{Soluble Protein}}{\text{Total Whey Protein}} \times 100$ (%)
-	-	UWPC	91.50
1.5	Acid	UWPC	38.60
3.0	Acid	UWPC	42.00
4.5	Acid	UWPC	42.00
1.5	Rennet	UWPC	49.90
3.0	Rennet	UWPC	44.30
4.5	Rennet	UWPC	50.40
-	-	DWPC	25.18
1.5	Acid	DWPC	25.47
3.0	Acid	DWPC	26.43
4.5	Acid	DWPC	26.00
1.5	Rennet	DWPC	27.00
3.0	Rennet	DWPC	26.63
4.5	Rennet	DWPC	24.00

Figure 4. Meltability of rennet and acid casein model process cheese. Volumetric values for acid casein model cheeses represent added 5 N NaOH per 2 kg.

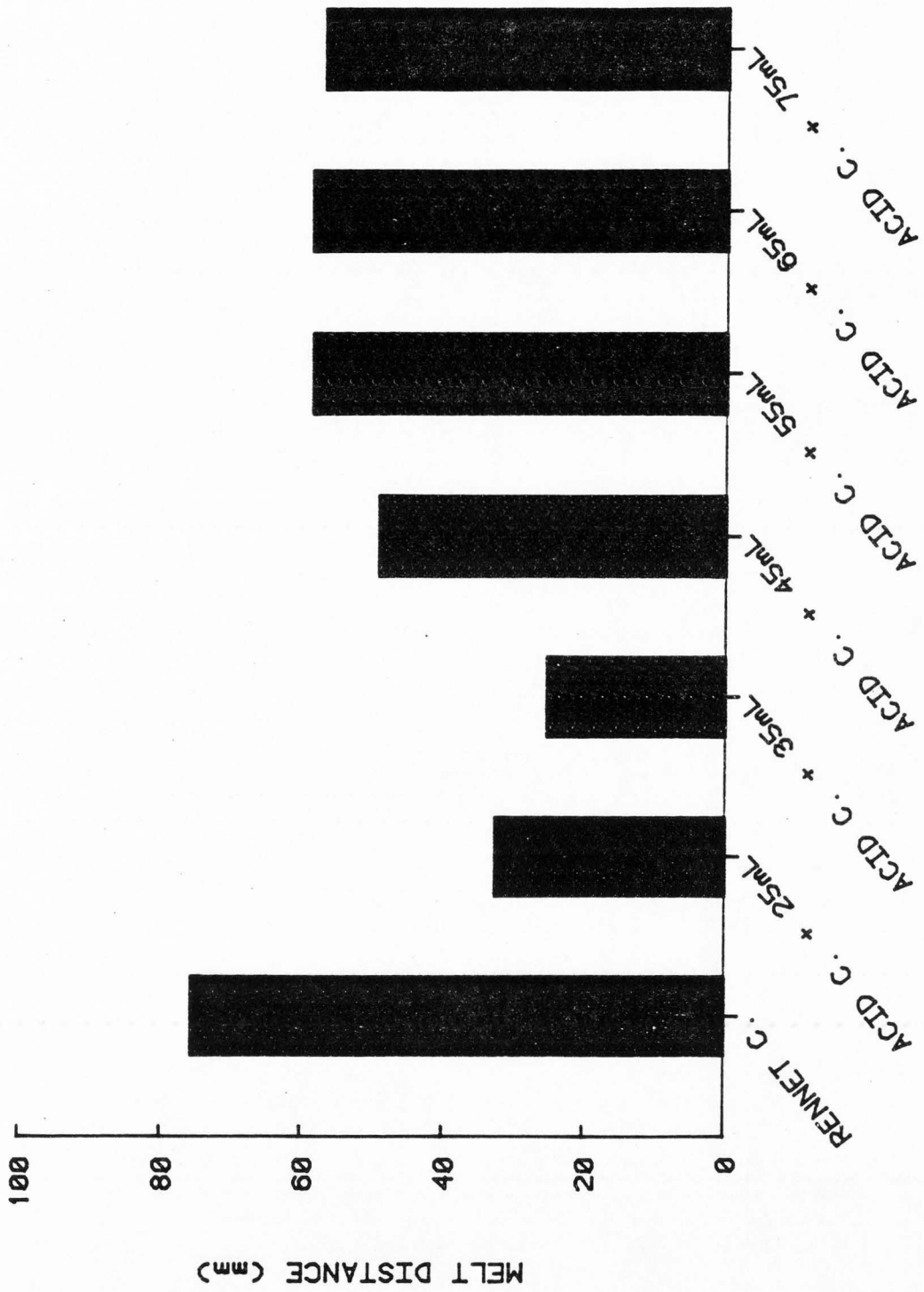
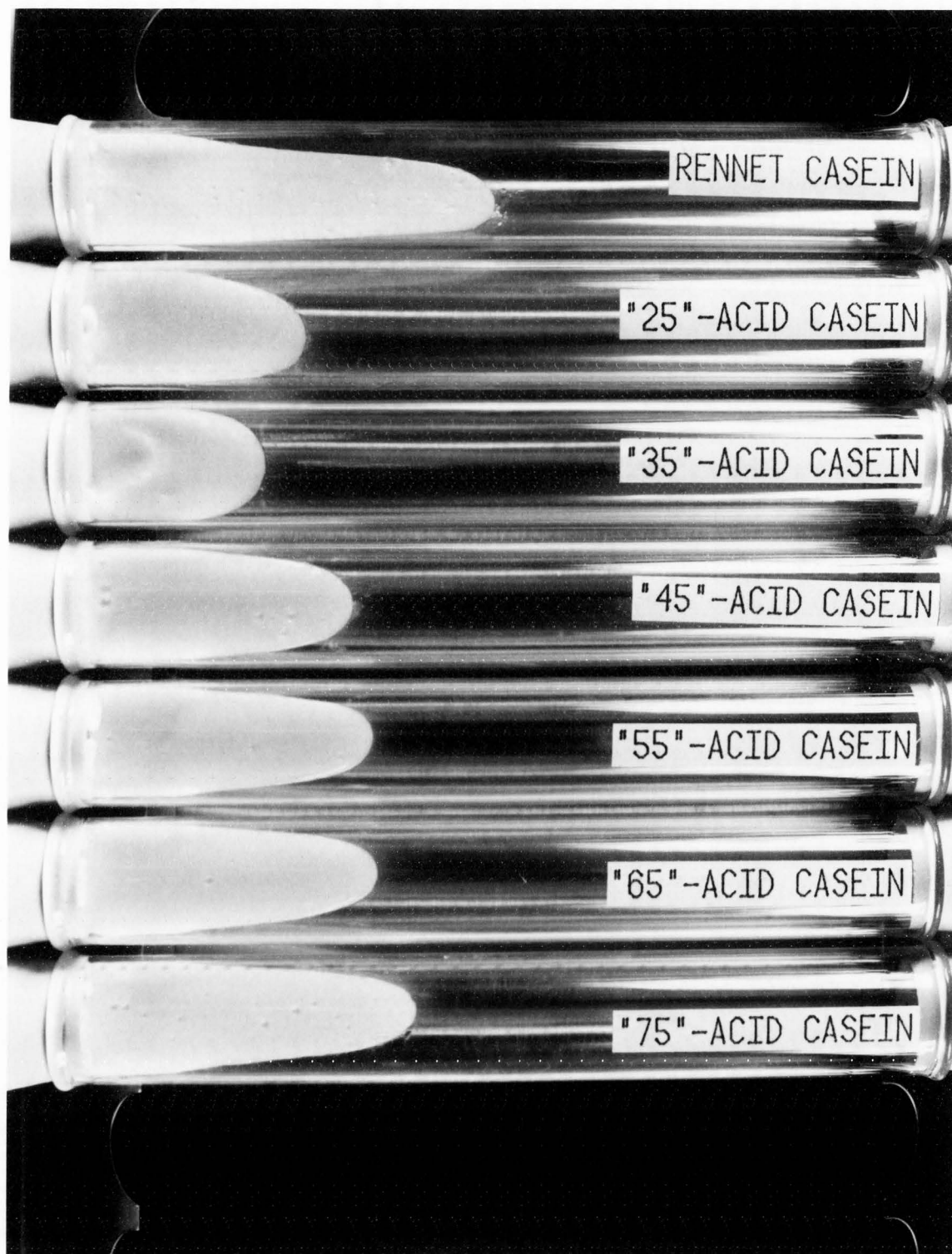


Figure 5. Meltability of model process cheese made with rennet casein compared to casein containing 25, 35, 45, 55, 65 and 75 mL 5 N NaOH per 2 kg followed by adjustment to pH 5.7 with lactic acid.



protein solubility (at pH 4.40) beyond that produced by the initial heat treatment of the whey protein concentrate (75%).

Meltability of Model Process Cheese

The relationship between meltability of model process cheese and the type of casein used is depicted in Figures 4 and 5. The rennet casein model melted significantly better than the acid casein models. As the alkaline treatment of acid casein increased (by increasing the amount of NaOH), the meltability increased to a maximum. Increasing the pH environment of the acid casein process cheese blend by increasing the level of NaOH solubilizes the acid casein. Increased solubilization of the acid casein may loosen the structure of the process cheese permitting enhanced cheese flow during the meltability test. There was no further increase in cheese flow when greater than 55 mL 5 N NaOH per 2 kg was used to condition the acid casein model.

Acid casein models conditioned with 35 mL 5 N NaOH per 2 kg had less cheese flow than when conditioned with 25 mL 5 N NaOH per 2 kg. This decrease in meltability was repeatedly observed and measured but remains unexplained.

Appendix C records the mean \pm standard deviation values and corresponding analysis of variance tables of meltability data of model process cheese. Tables 15 and 16 of Appendix C indicate that significant differences occurred between

Figure 6. Meltability of model process cheese with different emulsifying salts.

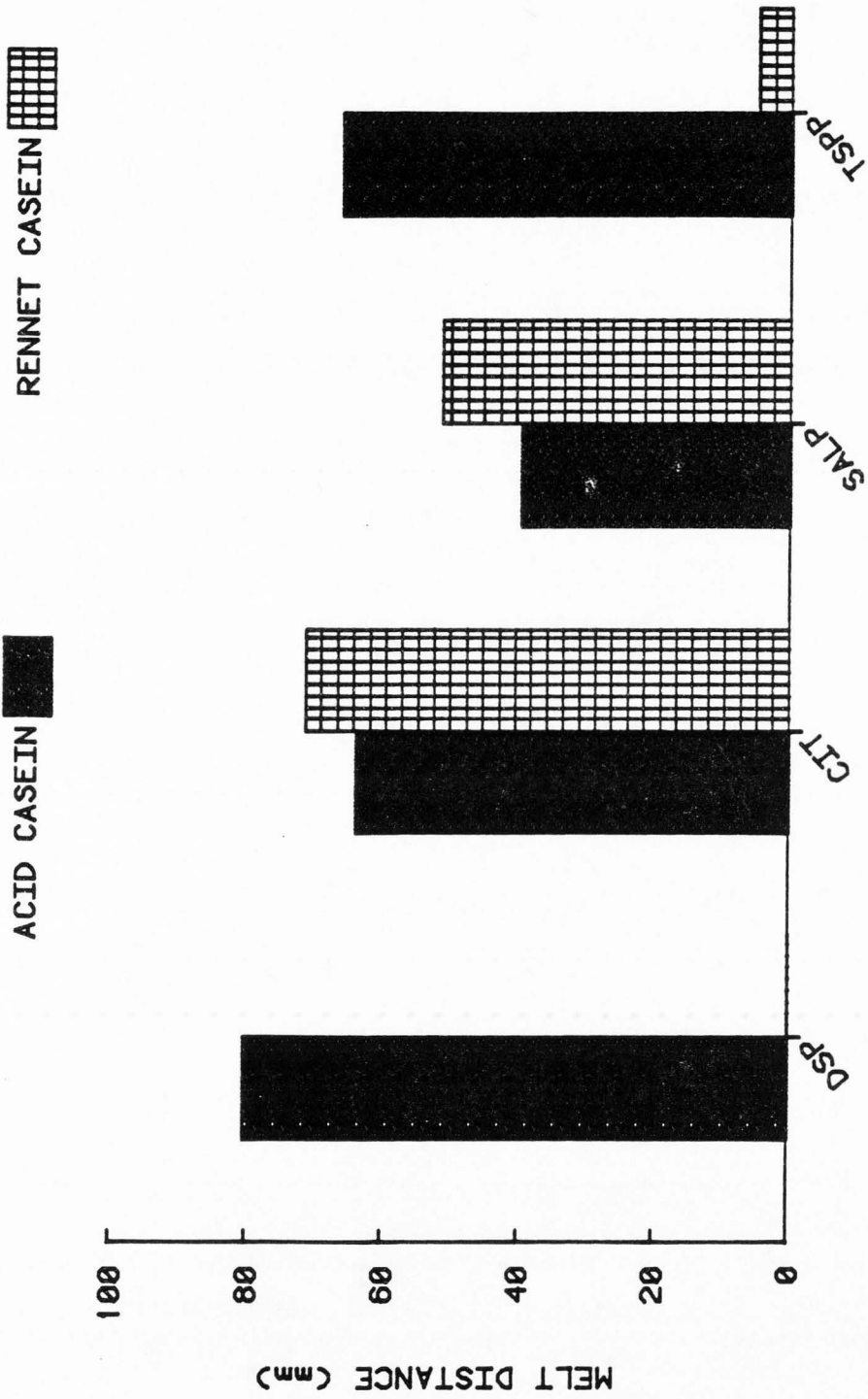
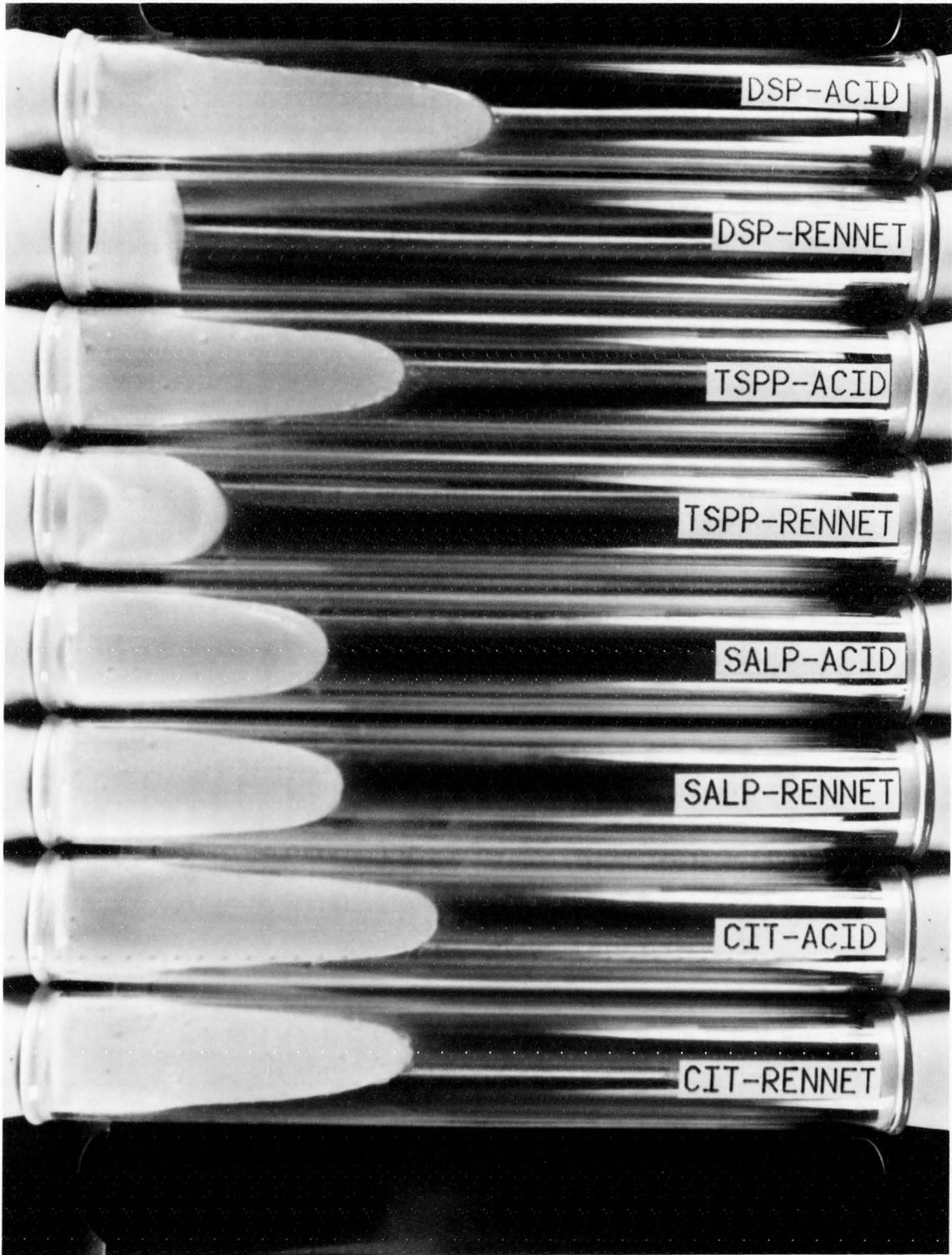


Figure 7. Meltability of model process cheese with different emulsifying salts (acid casein models conditioned with NaOH).



samples: rennet casein model and acid casein models with 25, 35, or 45 mL 5 N NaOH per 2 kg added were all statistically different at $P=.05$. The acid casein models with 55, 65, or 75 mL 5 N NaOH per 2 kg were not statistically different at the same probability level.

Meltability of Model Process Cheese
with Different Emulsifying Salts

There were dramatic differences in meltability between rennet and acid casein model process cheeses when different emulsifying salts were used in the formulations (Figures 6 and 7). Emulsifying salts DSP and TSPP resulted in the greatest difference between the two model cheeses. There was no melting of the rennet casein models when these salts were used whereas the acid casein models (NaOH conditioned) melted 80 and 70 mm with DSP and TSPP, respectively.

Poor meltability of rennet casein cheese with TSPP suggests that this emulsifying salt does not chelate sufficient calcium from the rennet casein and, therefore, prohibits melt. This emulsifying salt produces a process cheese similar to one prepared with DSP: a firm protein matrix with no meltability.

Rennet casein model process cheese melts best with CIT as the emulsifying salt (Fig. 6). The effectiveness of CIT in producing process cheese with good melting quality has been shown by Templeton and Sommer (47), Rayan et al. (38),

and Thomas et al. (49). CIT complexes with micellar calcium phosphate causing disaggregation of the casein micelles (29,33) thereby loosening the protein structure sufficiently to allow cheese flow.

Rennet casein model process cheese prepared with SALP melts slightly less than cheese prepared with CIT. The actual process by which SALP enhances meltability is not indicated in the literature but it can be theorized that SALP chelates the casein-bound calcium similar to CIT. The subsequent disaggregation produces a less structured protein matrix of cheese allowing a meltable product. Rayan et al. (38) showed that emulsification by SALP was slower than with CIT. The former salt required more time in the cooker to effect more complete emulsification.

Acid casein model process cheeses prepared with various emulsifying salts require a different explanation. No calcium is bound to casein at the isoelectric point (pH 4.6) of this protein (60). Atomic absorption measurement for calcium in the acid casein indicates that this salt was not present (Table 1). Thus, calcium complexing or chelating by CIT or SALP does not occur.

Acid casein cheeses with CIT or SALP do not melt as well as rennet casein cheeses with these emulsifying salts. The acid casein cheeses are well emulsified as there is no oiling-off in the melted samples (Fig. 7). Thus, the mechanism by which CIT or SALP provide for emulsification of

acid casein process cheese is not the same as with rennet casein cheese.

Similarly, DSP or TSPP produces acid casein process cheese with excellent melt (Fig. 6). This is in sharp contrast to the rennet casein cheeses prepared with these emulsifying salts.

The conditioning step of acid casein with NaOH and an emulsifying salt may explain the meltability of the acid casein cheeses. Each salt influences the conditioning pH of the blend in the cooker (Table 3) and, thereby, the extent of solubilization of the casein. The solubilization of the acid casein in this manner may be similar to the disaggregation of rennet casein by calcium removal with subsequent destabilization of rennet casein particles.

Acid casein cheeses with DSP and TSPP were conditioned at high pH levels (7.55 and 7.75, respectively). These pH levels may have solubilized sufficient acid casein resulting in a very loose protein matrix around the fat globules and, subsequently, process cheeses with good meltability.

This reasoning, however, fails to explain the lower meltability of acid casein cheese with SALP. This emulsifying salt causes a conditioning pH of 7.65, comparable to cheeses prepared with DSP and TSPP. Conditioning pH and its effective solubilization of acid casein may only partly explain the action of emulsifying salt on acid casein. Other factors, such as phosphate

binding or sodium displacement of hydrogen to casein may influence the final protein-fat emulsification and structure of acid casein process cheese.

Meltability of Model Process Cheese with
Added Whey Protein Concentrate

Meltability of process cheese models decreased as the concentration of WP increased (Figures 8 and 9; Tables 19-22, Appendix C). UWPC added to the acid casein models (NaOH conditioned) (Fig. 8) decreased cheese flow linearly with increasing WP addition. This confirms the patent by Schulz (42) describing a method for producing a melt resistant process cheese. The process involved the incorporation of 3-7% (w/w) of a coagulable protein, such as milk albumin or lactalbumin. Heat-induced gelation of the protein when process cheese was tested for meltability was implied in the patent.

There were no body defects or loss of emulsification in the acid casein cheeses (Fig. 11). UWPC added to the rennet casein models decreased cheese flow and severely affected the body of the cheese. Moderate oiling off was noted in the sample with 1.5% UWPC. Severe oiling off and a very porous surface were defects observed in the samples containing 3.0 and 4.5% UWPC (Fig. 13). The latter sample collapsed and slid in the melting tube rather than exhibiting a true melting property. This defect may account

Figure 8. Meltability of acid (■) and rennet (▲) casein model process cheese with added undenatured whey protein concentrate.

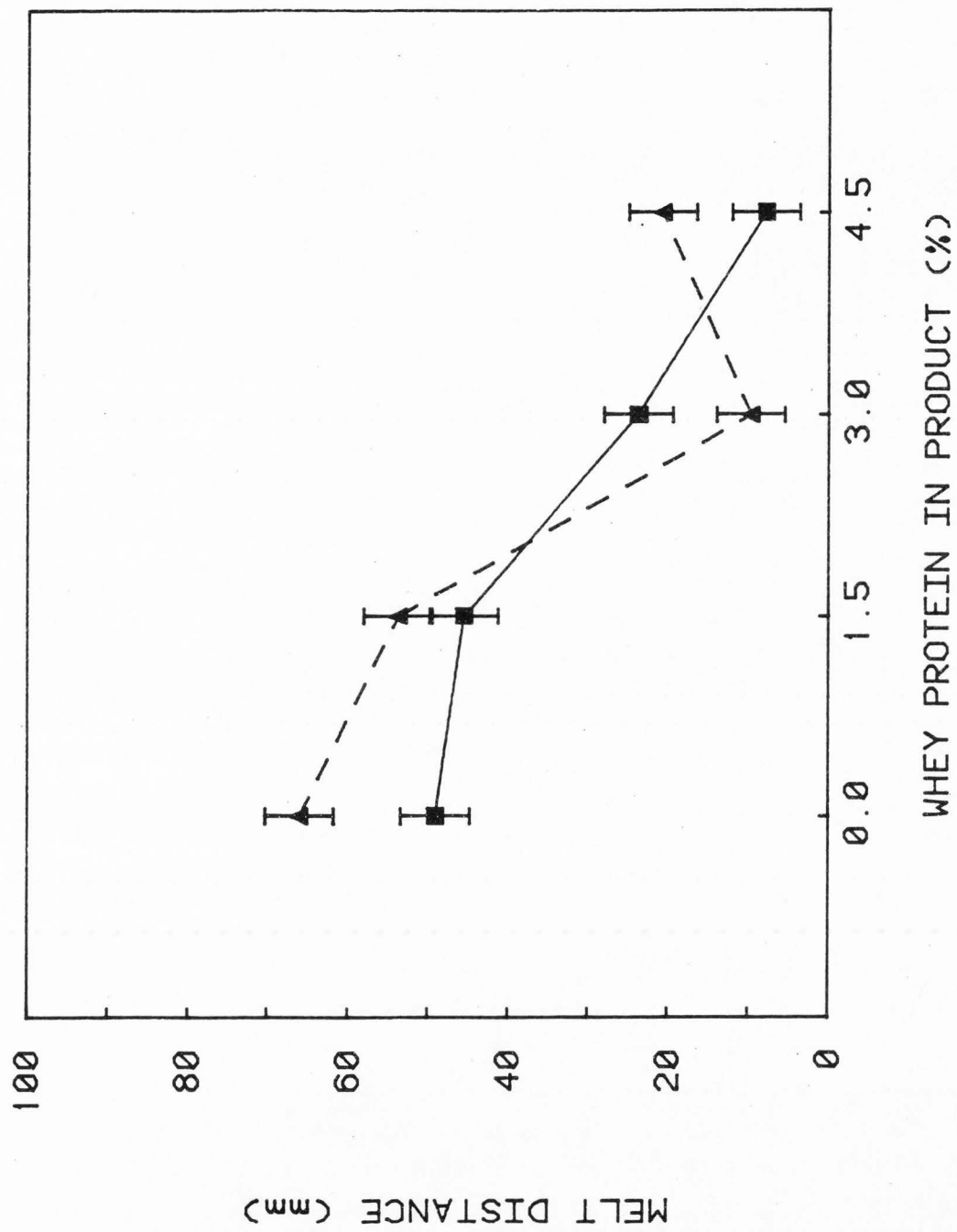


Figure 9. Meltability of acid (■) and rennet (▲) casein model process cheese with added denatured whey protein concentrate.

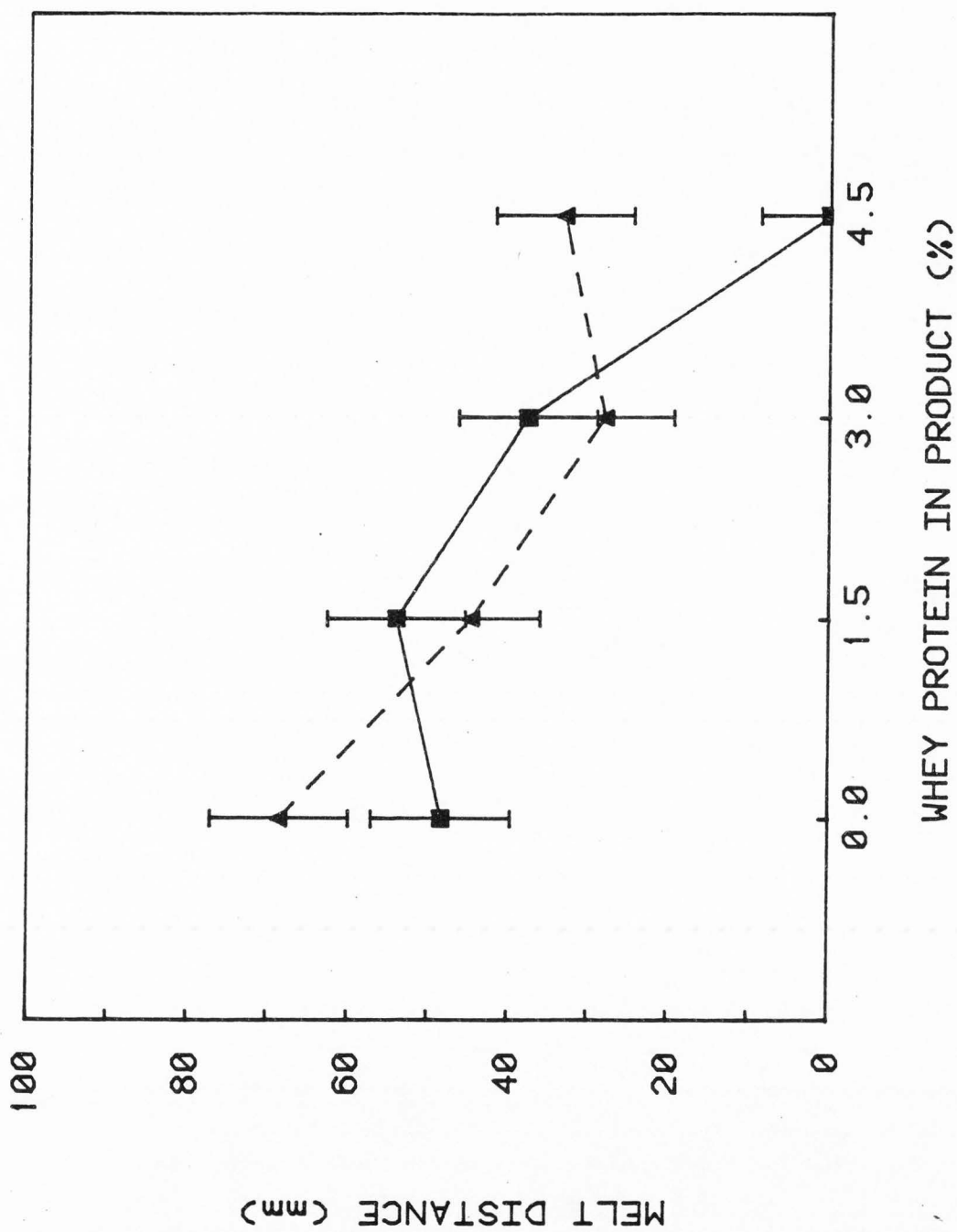


Figure 10. Meltability of acid casein model process cheese with added undenatured (—) and denatured (---) whey protein concentrate.

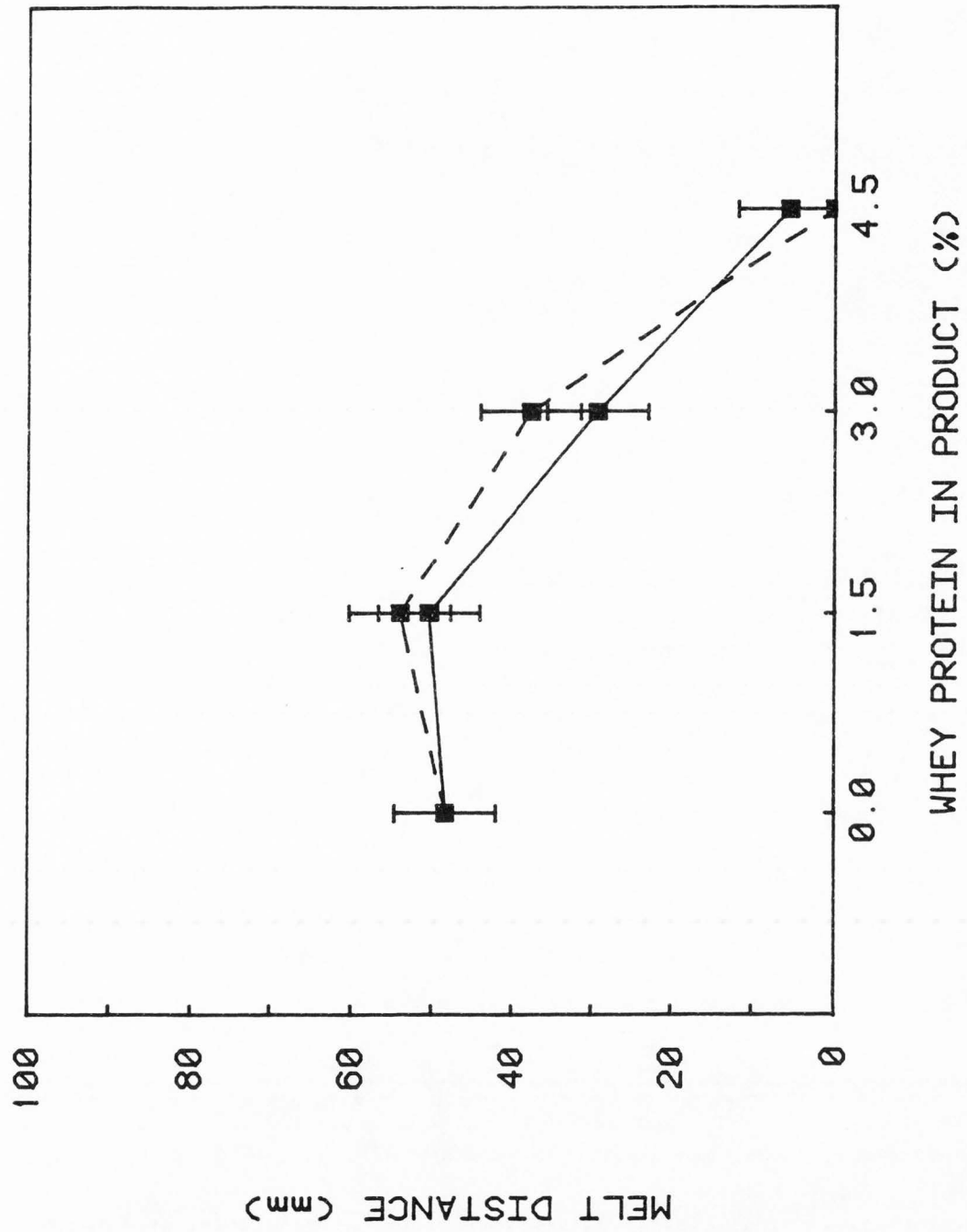


Figure 11. Meltability of acid casein model process cheese with added undenatured (N) and denatured (D) whey protein concentrate.



for the apparent increase in meltability when UWPC concentration was increased from 3.0 to 4.5% (Fig. 8).

DWPC added to acid casein models at 1.5 and 3.0% levels did not decrease the meltability as much as in the rennet model (Fig. 9). At the 4.5% addition the acid casein cheese did not melt whereas the corresponding rennet casein model displayed increased meltability. This apparently greater cheese flow with 4.5% DWPC resulted more from the change in cheese body previously mentioned. The oiling off defect and porous surface structure at the highest DWPC addition to the rennet model were as pronounced as in the UWPC model cheeses.

The meltability of the acid casein models made with either UWPC or DWPC (Figures 10 and 11) showed clearly that heat treatment of the WPC did not affect cheese flow. There was no significant difference between meltabilities of acid casein cheese models at any given whey protein level whether the whey protein was UWPC or DWPC (Tables 23 and 24, Appendix C).

The meltability of rennet casein models with either UWPC or DWPC added (Figures 12 and 13) also indicated that heat treatment of the WPC did not affect the cheese flow. At any given whey protein level there was no significant difference in meltability whether the whey protein source was UWPC or DWPC (Tables 25 and 26, Appendix C).

Figure 12. Meltability of rennet casein model process cheese with added undenatured (—) and denatured (---) whey protein concentrate.

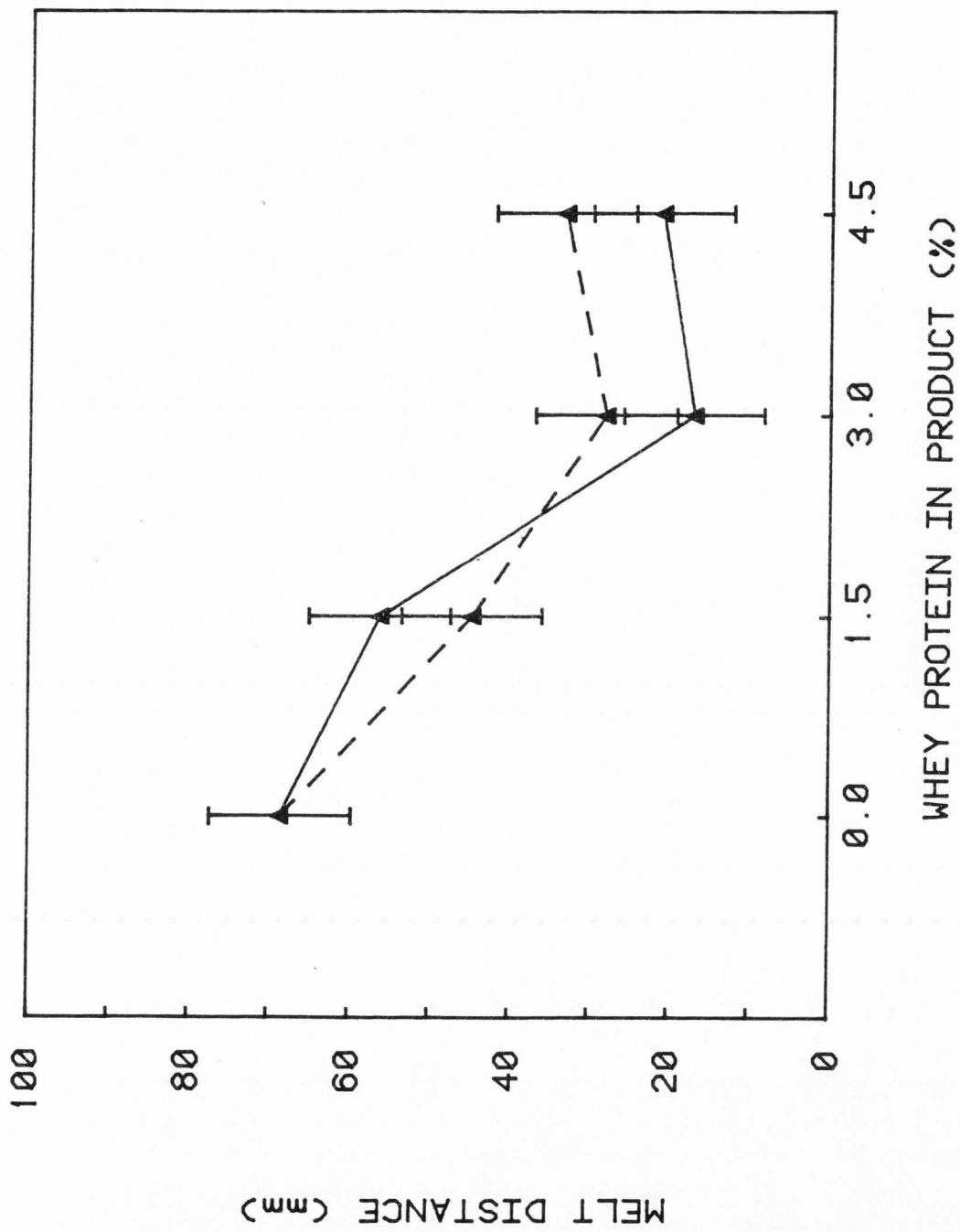
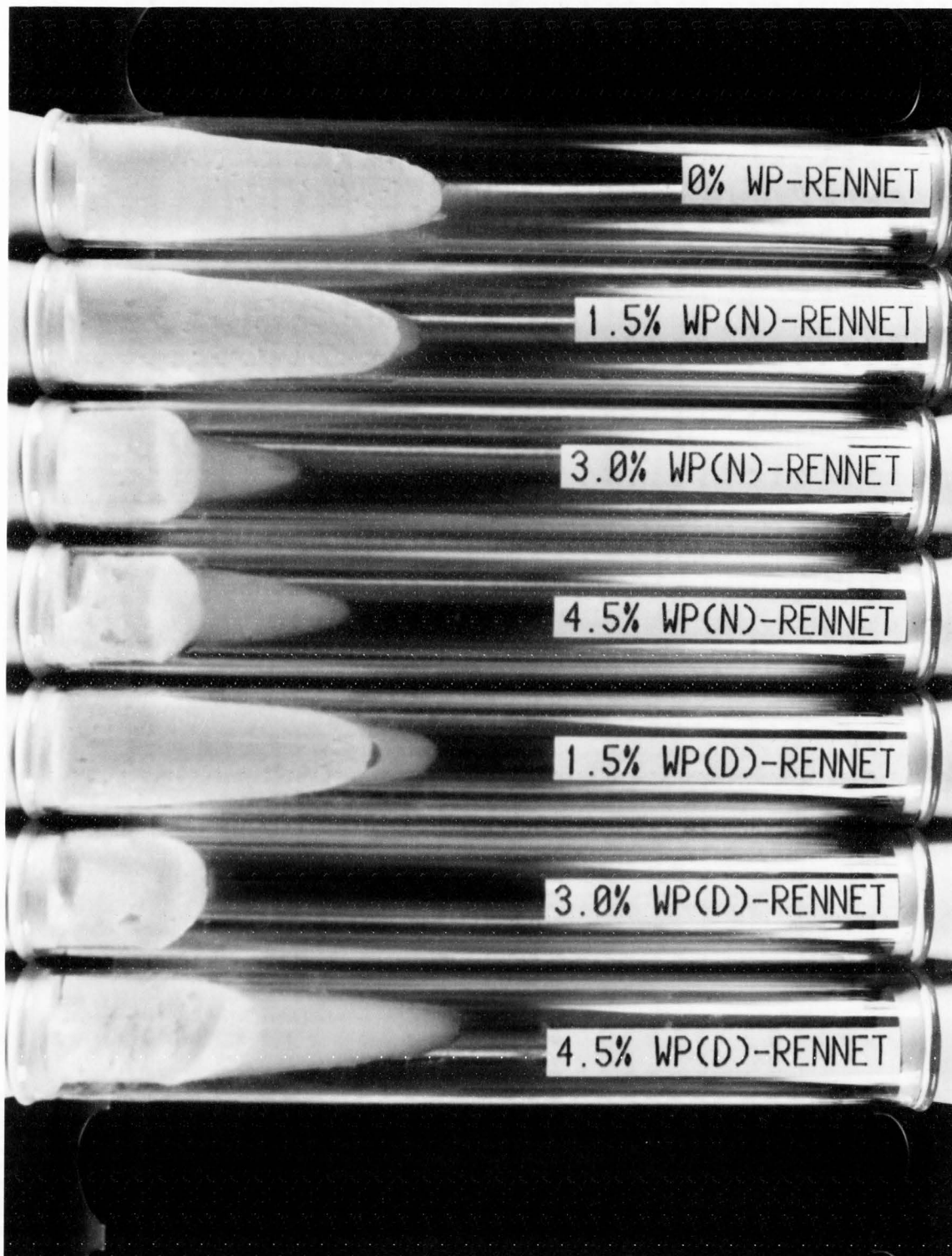


Figure 13. Meltability of rennet casein model process cheese with added undenatured (N) and denatured (D) whey protein concentrate.



Meltability of Rennet Casein Process

Cheese with Added Disodium Oxalate

Rennet casein cheese prepared with TSPP or DSP as emulsifying salt did not melt (Figures 6 and 7) whereas acid casein cheeses with the same emulsifying salts exhibited excellent melting properties. One important difference between rennet and acid casein is the calcium content (Table 1). It was theorized that TSPP and DSP did not bind sufficient calcium in the rennet casein which resulted in the serious lack of melting quality.

Rennet casein models made with TSPP or DSP and with added disodium oxalate melted well (Table 11 and Fig. 14). These results support the theory that orthophosphates and pyrophosphates lack adequate capacity to chelate calcium bound to casein. Oxalate is a strong calcium binding agent that in sufficient concentration can chelate all the calcium in milk (33). Oxalate binds the calcium that DSP and TSPP cannot, producing rennet casein model process cheeses with superior meltability (Fig. 14).

Rheology Measurements

Rennet and Acid Casein Process Cheese

Acid casein models were firmer than rennet casein model cheese (Fig. 15). Cheeses became firmer as the level of NaOH conditioning of acid casein increased. Acid casein

Table 11. Meltability¹ of TSPP and DSP rennet casein model process cheese with disodium oxalate as a calcium binding agent.

Sample		
Emulsifying Salt	Oxalate Added	Melt Distance ² (mm)
TSPP	No	13.7 ± 1.15 ^a
TSPP	Yes	94.0 ± 5.29 ^b
DSP	No	0.0 ± 0.0 ^c
DSP	Yes	61.0 ± 1.73 ^d

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P=.05.

Figure 14. Meltability of TSP and DSP rennet casein model process cheese with disodium oxalate added as a calcium binding agent.

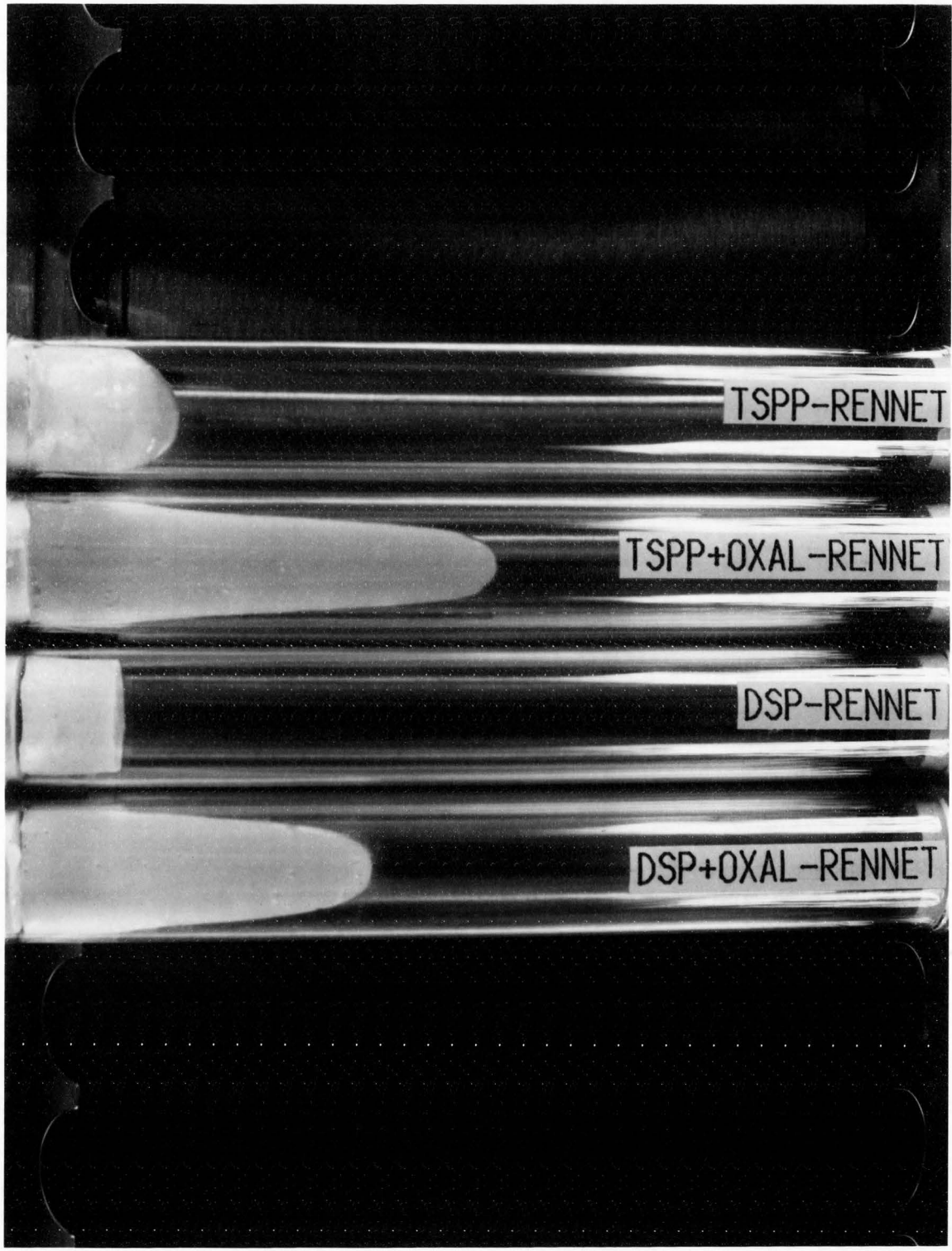


Figure 15. Firmness of rennet and acid casein model process cheese. Volumetric values for acid casein model cheeses represent added 5 N NaOH per 2 kg.

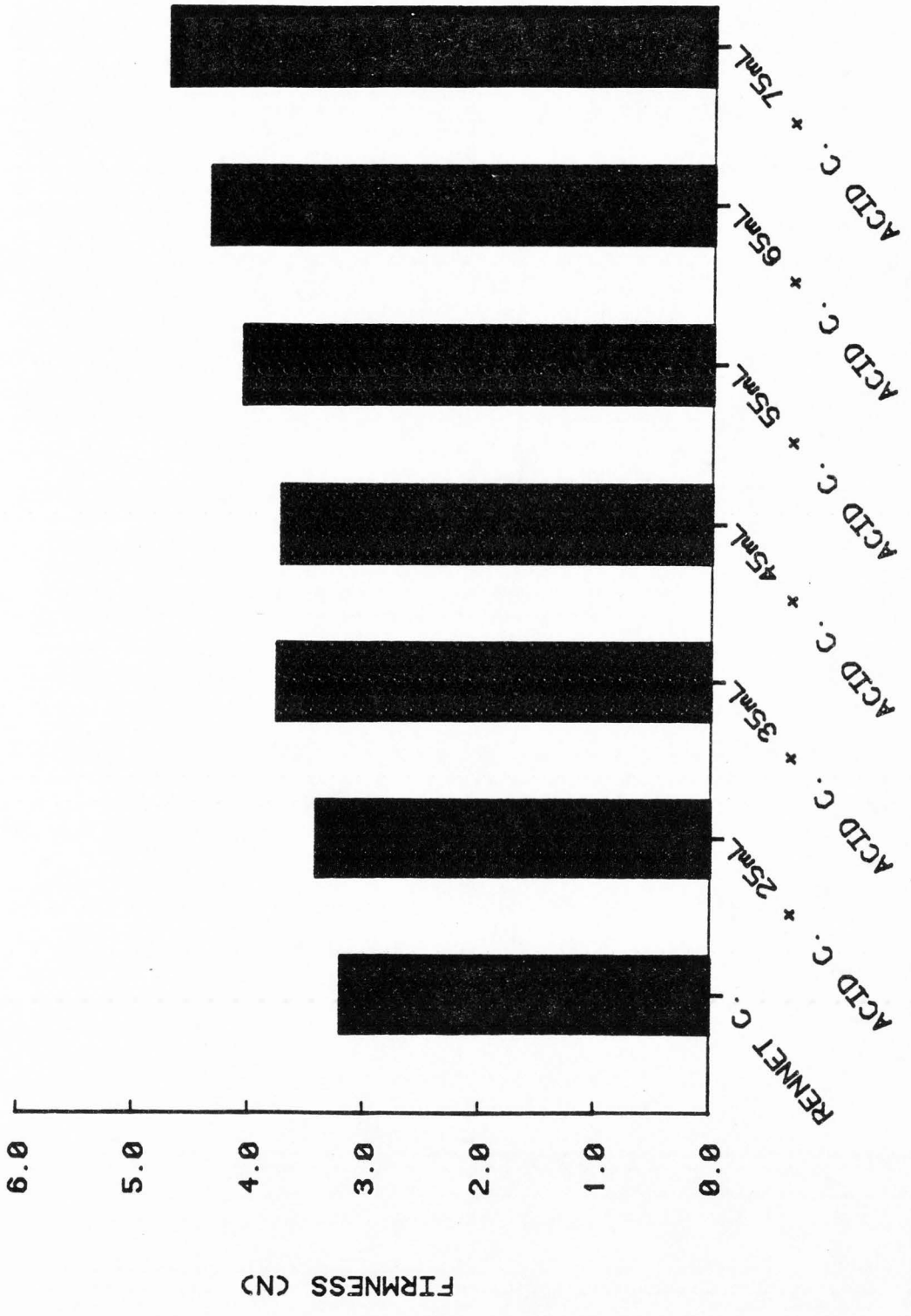
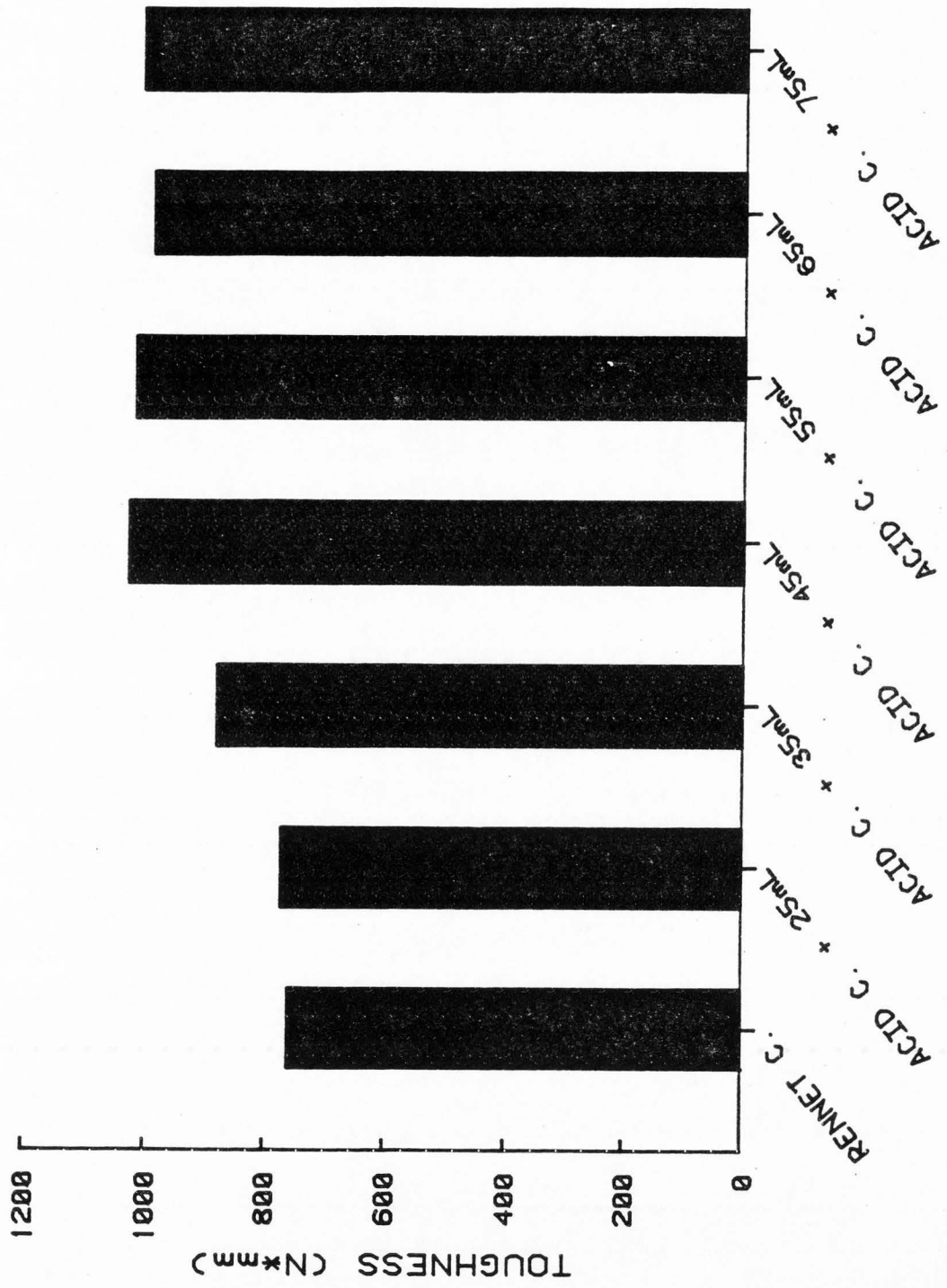


Figure 16. Toughness of rennet and acid casein model process cheese. Volumetric values for acid casein model cheeses represent added 5 N NaOH per 2 kg.



models prepared with 35 and 45 mL of NaOH per 2 kg were not statistically different ($P=.05$) whereas all other samples showed a significant difference in firmness (Tables 27 and 28, Appendix C). The same trend occurred with toughness measurements (Fig. 16), albeit the statistical difference of toughness among the samples was less clearly defined as the firmness measurements (Tables 27 and 29, Appendix C). Acid casein cheeses prepared with 35, 45, 55, 65 and 75 mL NaOH were not significantly different in firmness ($P=.05$).

The conditioning of acid casein by NaOH treatment affects the meltability and firmness of the process cheeses but does not alter the degree of toughness (rupture under compression) of the cheeses. No significant correlations occur between melt and firmness ($r=0.69$) nor melt and toughness ($r=0.48$) for conditioned acid casein cheese.

Rennet and Acid Casein Process Cheese Prepared with Different Emulsifying Salts

Acid casein models were firmer than the corresponding rennet casein cheese with the same emulsifying salt (Fig. 17). In both casein model cheeses, the decreasing order of firmness was TSPP, CIT, DSP and SALP (Tables 30 and 31, Appendix C).

Toughness of acid casein models with DSP, TSPP and CIT was not statistically different (Fig. 18; Tables 30 and 32, Appendix C). Acid casein model cheese prepared with SALP

Figure 17. Firmness of model process cheese with different emulsifying salts.

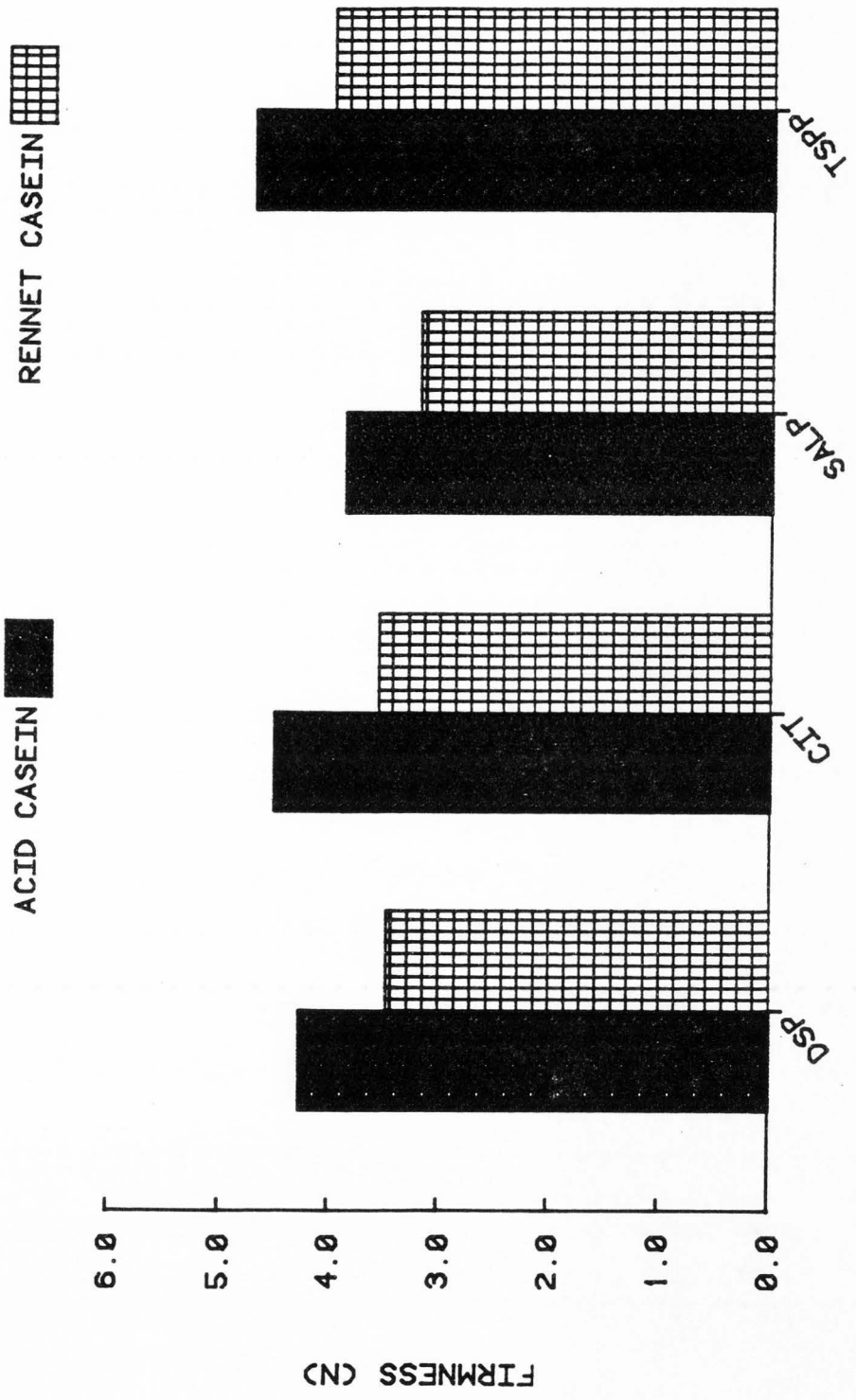
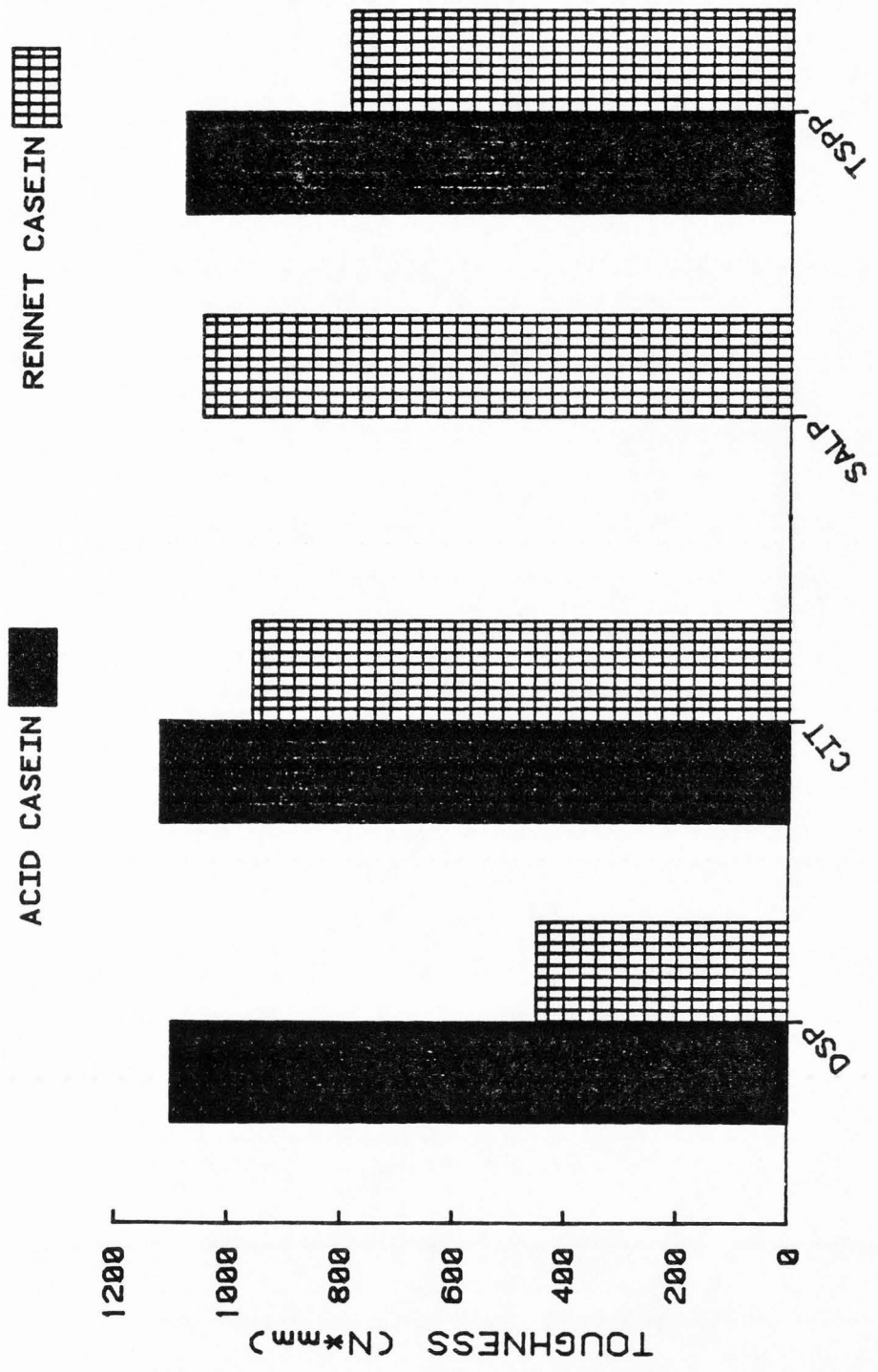


Figure 18. Toughness of model process cheese with different emulsifying salts.



was very soft (pliable) and did not rupture under compression. This was in sharp contrast to the rennet casein model prepared with SALP which was the toughest sample under compression followed by CIT, TSPP and DSP.

Rennet and Acid Casein Process
Cheese Prepared with Whey
Protein Concentrate

There were significant differences of firmness among the samples (Table 12). Analysis of the orthogonal contrasts selected indicated that four contrasts were significant at greater than $P=.05$ level. These contrasts included:

1. acid vs. rennet casein with WP absent;
2. acid vs. rennet casein with WP present;
3. undenatured vs. denatured WP in the formulation;
4. linear effect of DWP present in the acid casein cheeses.

The contrast of WP present vs. WP absent in cheeses was significant at the $P=.09$ level. When WP was present, however, there was no casein x WP type (UWP or DWP) interaction effect ($P=.17$).

Toughness measurements indicated numerous significant differences among the samples (Table 13):

1. absence vs. presence of WP;
2. acid vs rennet casein with WP absent or present;
3. linear effect of UWP or DWP in acid casein cheese;

Table 12. Analysis of variance of effect of type of casein and type of whey protein on firmness.

Source	df	SS	MS	F	Sig Level
Treatment	13	100.5301	7.7331	9.28	0.0001
WP Abs vs WP Pres	1	2.6908	2.6908	3.23	0.0939
Acid vs Ren, WP Abs	1	4.2001	4.2001	5.04	0.0414
Acid vs Ren, WP Pres	1	63.3616	63.3616	76.05	0.0001
Native vs Denat. WP	1	19.8916	19.8916	23.88	0.0002
Casein x WP Type	1	1.7600	1.7600	2.11	0.1681
Lin WP, Acid-Native	1	0.4056	0.4056	0.49	0.4968
Quad WP, Acid-Native	1	0.1964	0.1964	0.24	0.6349
Lin WP, Acid-Denat.	1	5.9203	5.9203	7.11	0.0185
Quad WP, Acid-Denat.	1	0.2134	0.2134	0.26	0.6206
Lin WP, Ren-Native	1	1.3443	1.3443	1.61	0.2247
Quad WP, Ren-Native	1	0.1721	0.1721	0.21	0.6564
Lin WP, Ren-Denat.	1	0.3553	0.3553	0.43	0.5243
Quad WP, Ren-Denat.	1	0.1869	0.1869	0.02	0.8831
Rep(Treatment)	14	11.6639	0.8331		
Sample(Rep)	56	5.0656	0.0905		
Deter(Sample)	84	3.5128	0.0418		
Total	167	120.7723			

Table 13. Analysis of variance of effect of type of casein and type of whey protein on toughness.

Source	df	SS	MS	F	Sig Level
Treatments	13	648264.8	498664.21	21.22	0.0005
WP Absent vs Pres	1	1079875.3	1079875.3	45.95	0.0001
Acid vs Ren, WP Abs	1	341381.3	341381.3	14.53	0.0025
Acid vs Ren, WP Pres	1	3678330.9	3678330.9	156.52	0.0001
Native vs Denat. WP	1	17133.5	17133.5	0.729	0.4000
Casein x WP Type	1	91191.6	91191.6	3.88	0.0750
Lin WP, Acid-Native	1	511046.4	511046.4	21.75	0.0005
Quad WP, Acid-Native	1	101444.4	101444.4	4.32	0.0600
Lin WP, Acid-Denat.	1	115748.3	115748.3	4.93	0.0400
Quad WP, Acid-Denat.	1	69.19	69.19	0.0029	0.0001
Lin WP, Ren-Native	1	202771.4	202771.4	8.63	0.0100
Quad WP, Ren-Native	1	7747.8	7747.8	0.330	0.6500
Lin WP, Ren-Denat.	1	331994.7	331994.7	14.13	0.0025
Quad WP, Ren-Denat.	1	3900.0	3900.0	0.166	0.7000
Rep(Treatment)	14	329009.5	23500.7	4.626	0.0500
Error	56	284504.8	5080.4		
Total	83	7096149.0			

4. a more strongly quadratic effect of DWP in acid casein cheese;

5. linear effect of UWP or DWP in rennet casein cheese.

Acid casein models were firmer than rennet casein models at all levels of UWP addition (Fig. 19). Cheese firmness decreased when 1.5% UWP was added, but did not decrease further at 3.0% UWP. At 4.5% UWP, rennet casein model was less firm than at lower UWP levels, probably due to the loss of emulsion and open texture previously explained. The acid casein model with 4.5% UWP increased in firmness.

Cheese toughness decreased as UWP addition increased in both cheese models (Fig. 20). Acid casein models were significantly tougher than rennet casein models at corresponding UWP concentrations.

Adding DWP to rennet and acid casein models resulted in the same general trend of sample firmness and toughness as UWP addition. Acid casein models were firmer (Fig. 21) and tougher (Fig. 22) than rennet casein models at all levels of DWP added. Acid casein models increased in firmness as the level of added DWP increased whereas the rennet casein models decreased in firmness. Both casein cheese models decreased in toughness as the level of added DWP increased.



Figure 19. Firmness of acid (■) and rennet (▲)
casein model process cheese with
added undenatured whey protein
concentrate.

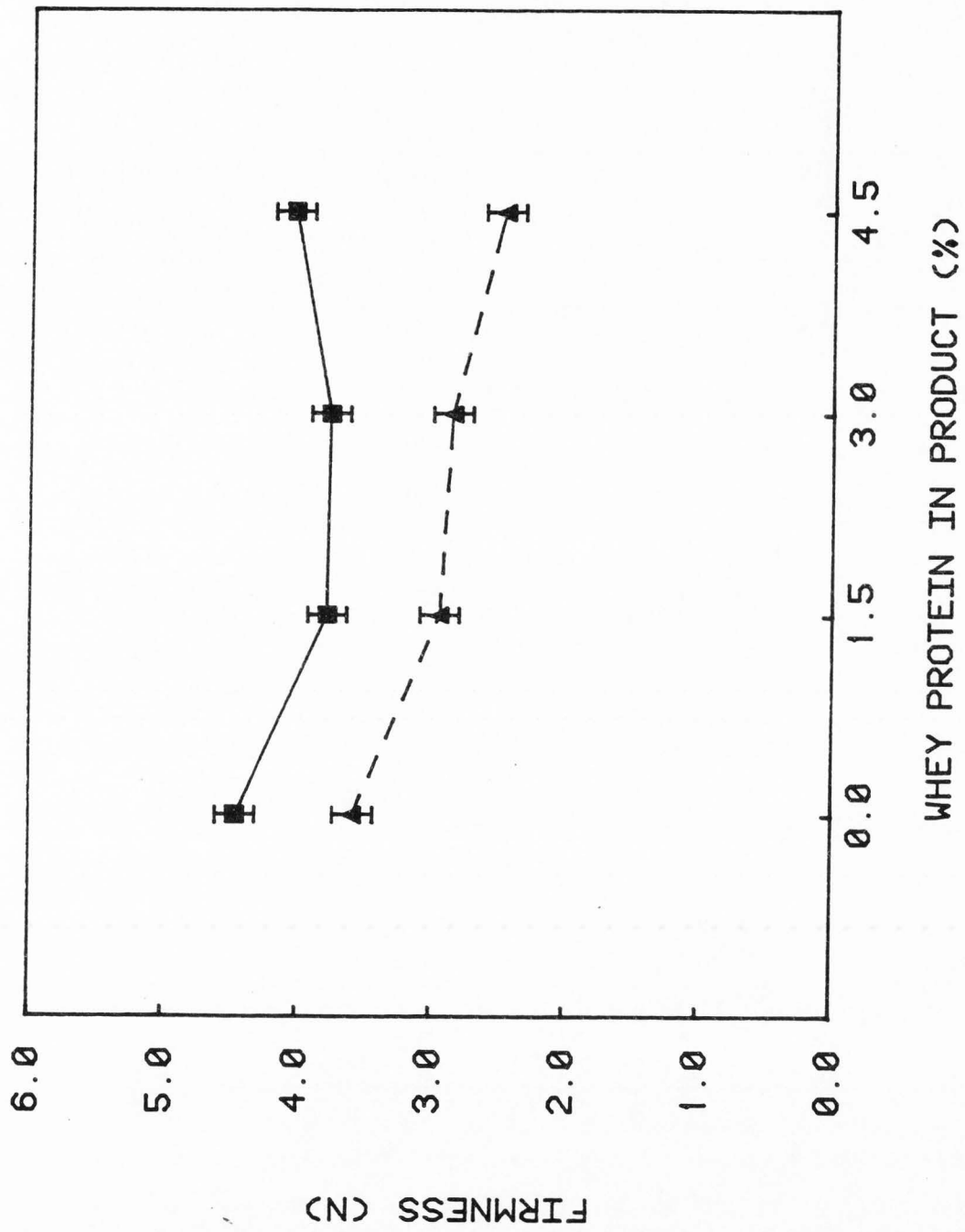




Figure 20. Toughness of acid (■) and rennet (▲)
casein model process cheese with
added undenatured whey protein
concentrate.

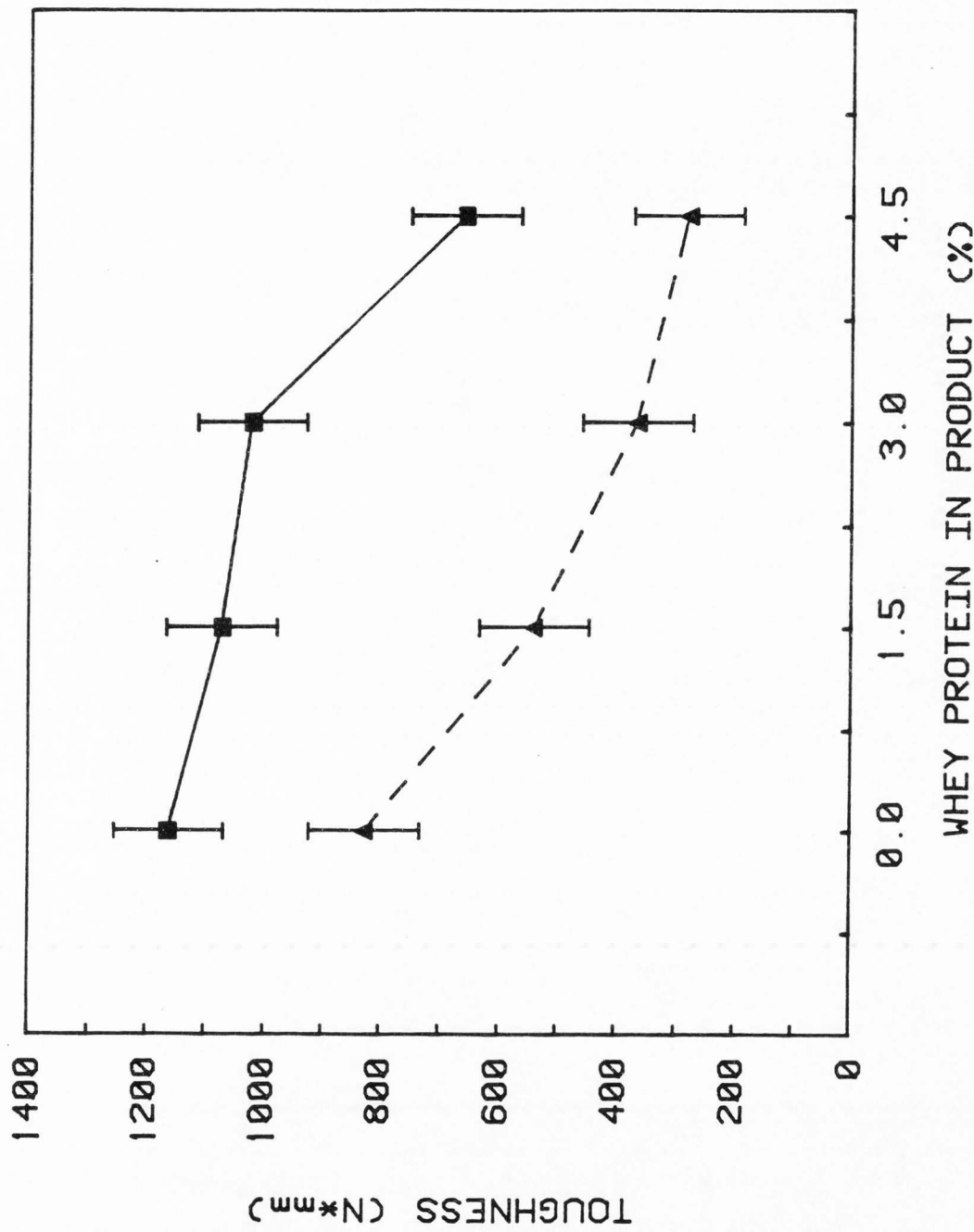


Figure 21. Firmness of acid (■) and rennet (▲)
casein model process cheese with
added denatured whey protein
concentrate.

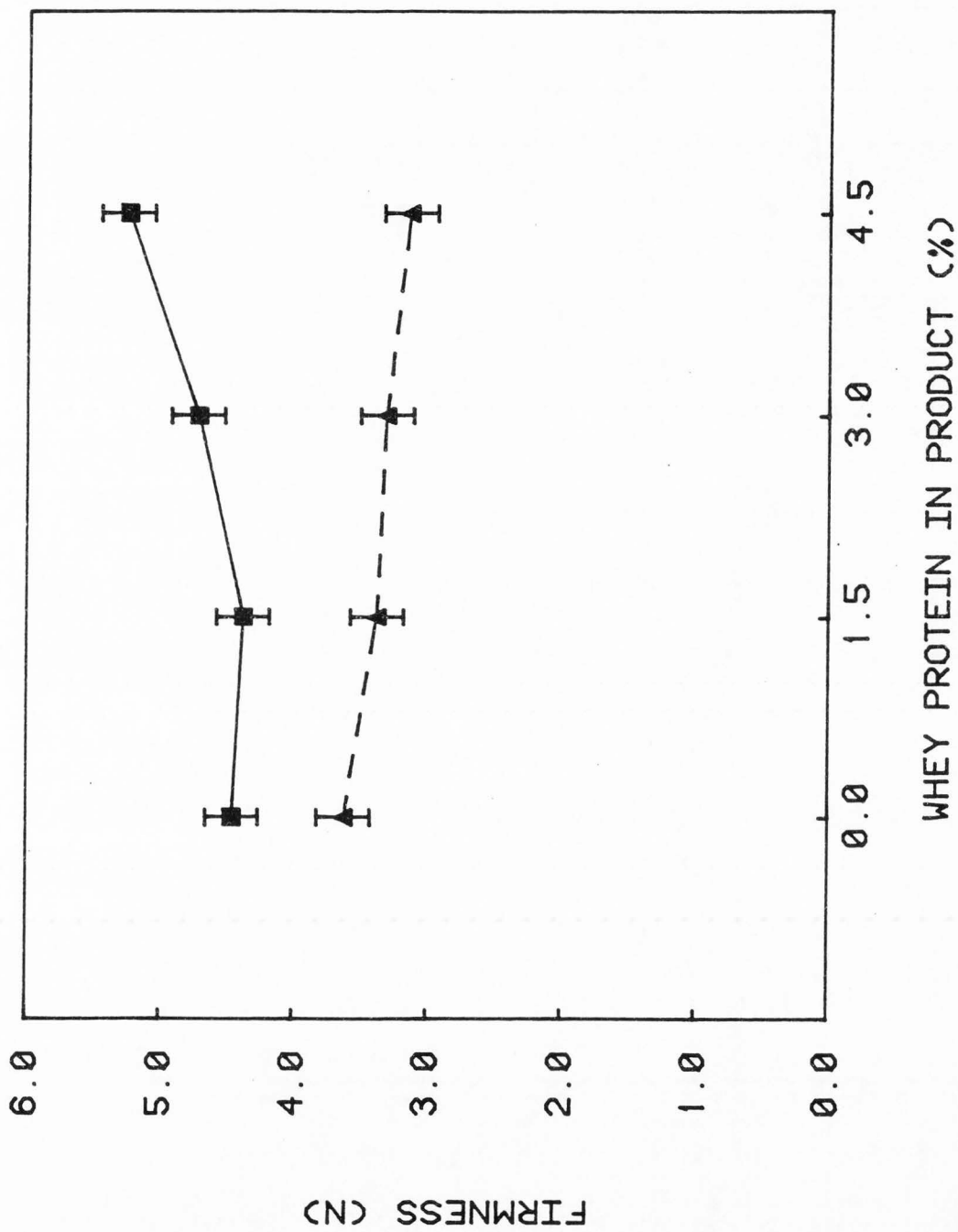
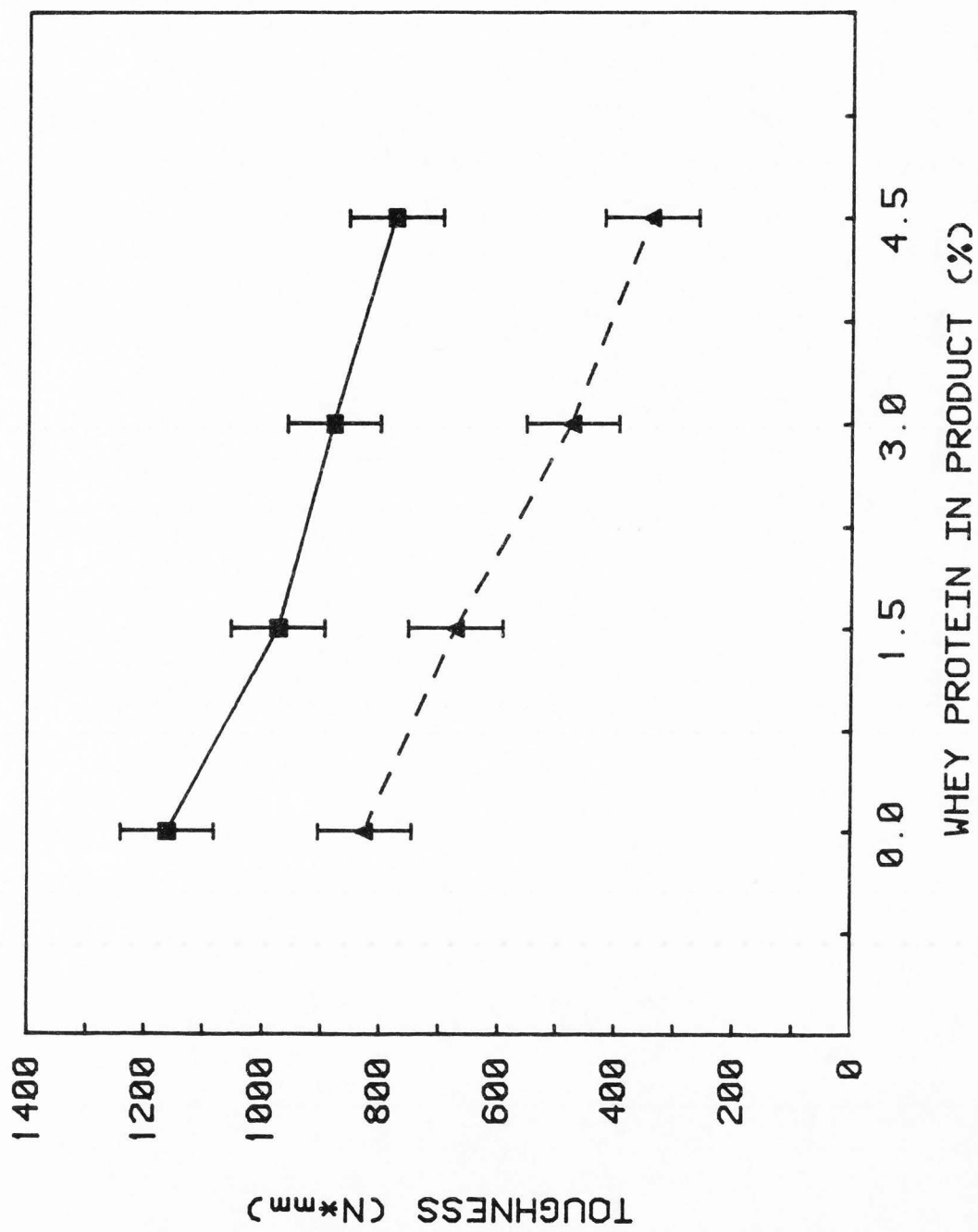


Figure 22. Toughness of acid (■) and rennet (▲)
casein model process cheese with
added denatured whey protein
concentrate.



If whey protein gelation causes decreased meltability as the protein concentration increases, it is reasonable to theorize that the three dimensional network of gelled whey protein would increase the toughness level of the cheese. The opposite is true, however. Both rennet and acid casein cheeses decrease in toughness significantly as the whey protein concentration increases (Table 33, Appendix C). The network of gelled whey protein has sufficient integrity to prevent cheese flow as well as to allow a decreased rupture point under compression.

If the network is considered brittle (thus permitting decreased yield point under compression) then toughness would be expected to decrease as noted in the present results. The impact of this brittle network on firmness is unclear as the rennet casein cheese decreases in firmness as whey protein concentration increases whereas the acid casein cheese with DWPC has an increasing firmness. The effect on cheese firmness by addition of UWPC to acid casein cheese does not indicate any clear trend.

Rennet Casein Process Cheese
with Disodium Oxalate as a
Calcium Binding Agent

Binding of calcium in the rennet casein process cheese model did not greatly affect the firmness and toughness of the sample when TSPP was the emulsifying salt (Table 14). The toughness level increased slightly with added oxalate.

When DSP was used as emulsifying agent the toughness of the sample with added oxalate increased substantially. The firmness level of cheese prepared with DSP (Table 14) did not change when oxalate was added.

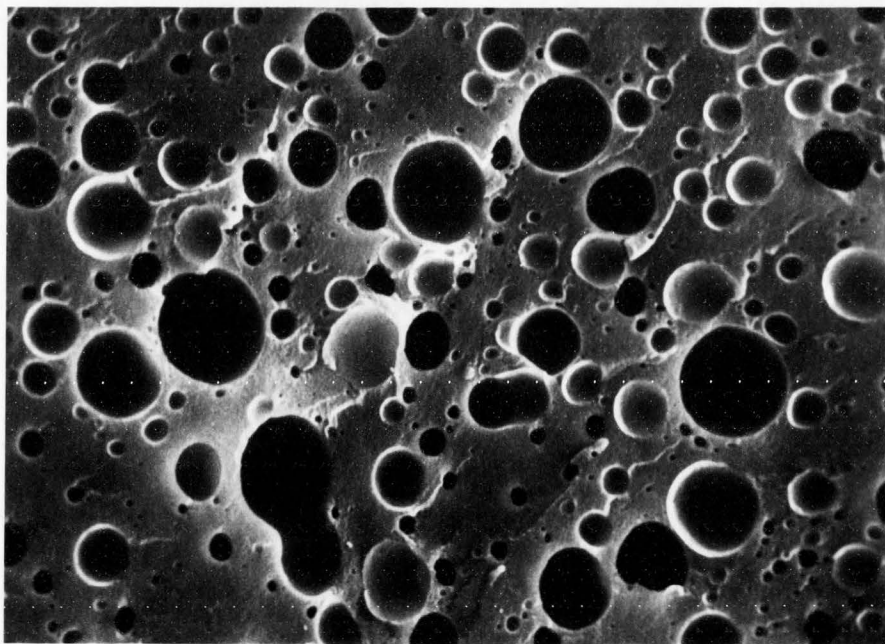
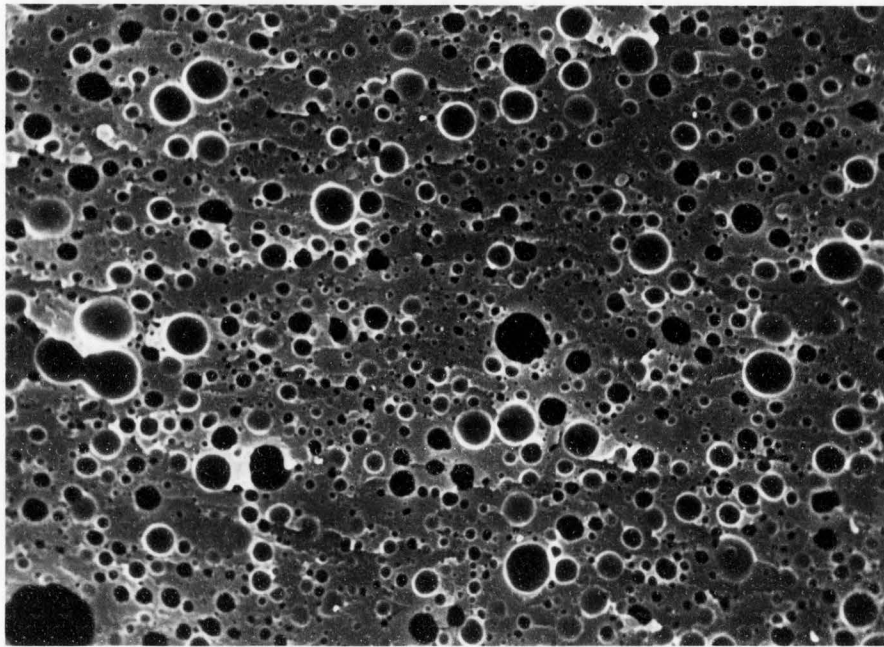
Table 14. Firmness and toughness of rennet casein process cheese with disodium oxalate as a calcium binding agent.

Sample	Firmness (N)	Toughness (N·mm)
TSPP	3.99 ± 0.15	790 ± 81
TSPP + Oxalate	3.73 ± 0.06	878 ± 39
DSP	3.47 ± 0.27	450 ± 91
DSP + Oxalate	3.36 ± 0.20	755 ± 87

Microstructure of Model Process Cheese

The microstructure of acid or rennet casein model process cheeses as viewed by scanning electron micrographs (SEM) aids in analysis of the differences in cheese meltability. Acid casein model cheeses conditioned with different levels of 5 N NaOH show markedly different degrees of emulsification. Acid casein cheese conditioned with 35 mL of 5 N NaOH per 2 kg has a fine emulsion (Fig. 23A) which may be responsible for its low meltability (25 mm). Acid casein cheese conditioned with 65 mL of 5 N NaOH per 2 kg

Figure 23. Scanning electron micrographs of pH conditioned acid casein process cheese.
A. with 35 mL 5 N NaOH per 2 kg;
B. with 65 mL 5 N NaOH per 2 kg.



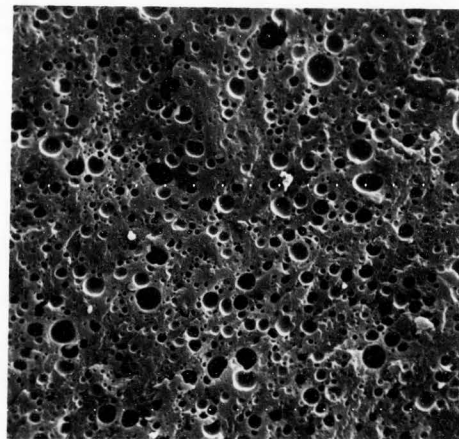
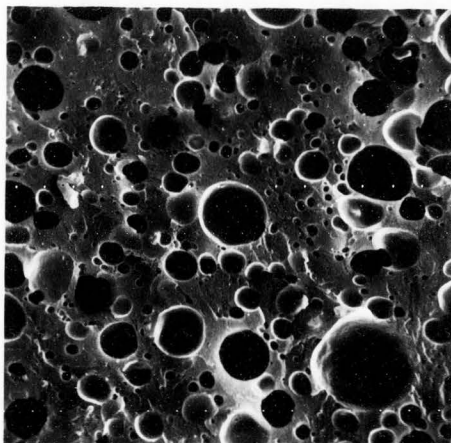
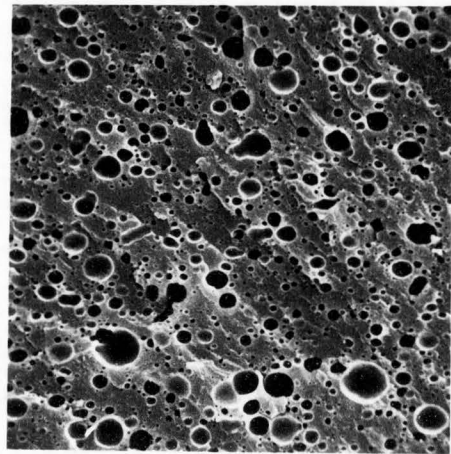
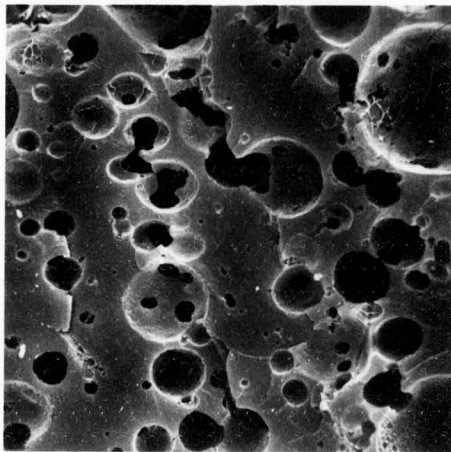
(Fig. 23B) has a more open structure (i.e. larger fat globule vacuoles in SEM photos) which may allow for greater meltability (58 mm). This correlation of emulsification extent and meltability closely agrees with Rayan et al. (38).

Rennet casein model process cheeses prepared with different emulsifying salts exhibit large differences in degree of emulsification. Cheese prepared with CIT has a very open structure with many differently sized fat globule spaces (Fig. 24A). A similar structure occurs in rennet casein cheese with SALP as the emulsifying agent. These cheeses have good meltability. Rennet casein cheese prepared with DSP, however, is extremely well emulsified (Fig. 24B) with very uniform size of small fat globule spaces. The rennet casein model cheese prepared with TSPP also has an extremely fine emulsification state. These cheeses do not display any meltability. The correlation between extent of emulsification and cheese meltability agrees well with previous work using natural process cheese (a close resemblance to the present rennet casein model process cheese) prepared with different emulsifying salts (38).

Rennet casein cheese prepared with DSP and added disodium oxalate displays good meltability and has a much more open microstructure than the comparable cheese prepared without added oxalate (Fig. 24B). The addition of oxalate to the cheese inhibits the over-emulsification which occurs

Figure 24. Scanning electron micrographs of model process cheese prepared with different emulsifying salts.

- A. Rennet casein cheese with CIT;
- B. Rennet casein cheese with DSP;
- C. Rennet casein cheese with DSP and disodium oxalate;
- D. Acid casein cheese with DSP.



in rennet casein cheese with DSP. Correspondingly, the less complete emulsification in the model cheese with DSP and oxalate allows greater meltability. Rennet casein cheese prepared with TSP and disodium oxalate also has larger fat droplets and exhibits greater cheese meltability than the corresponding model cheese without added oxalate.

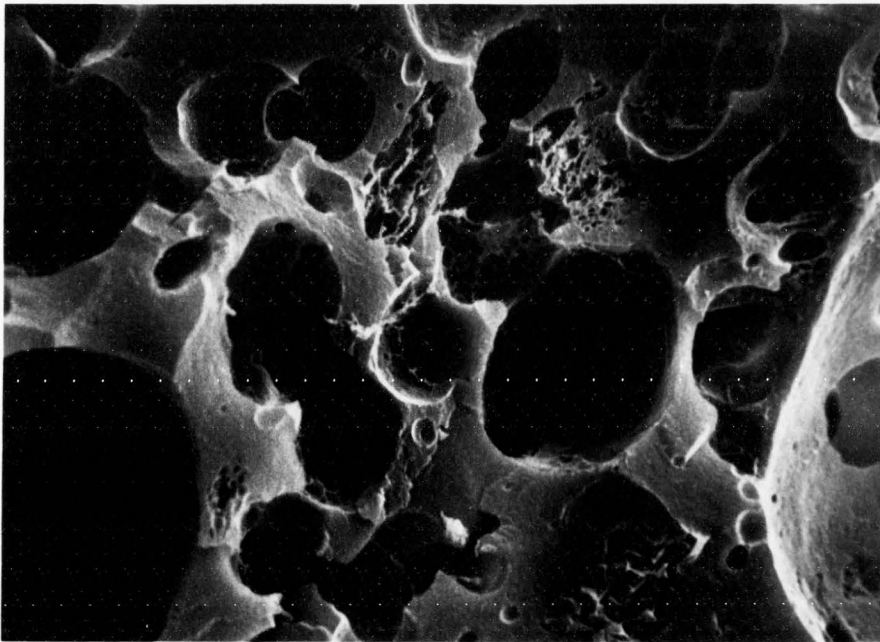
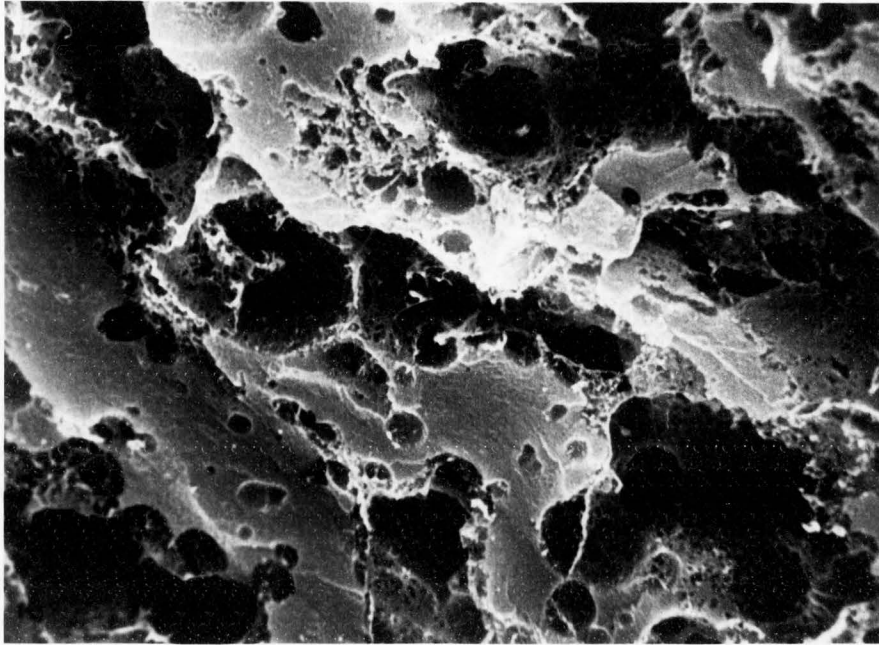
The correlation between emulsification extent and cheese meltability does not hold true for acid casein model process cheeses, however. Acid casein cheese prepared with DSP is very well emulsified (Fig. 24D) but displays excellent meltability (80 mm cheese flow).

The addition of whey protein concentrate to rennet casein model process cheese does not influence the degree of emulsification as the whey protein level increases. All rennet casein cheeses exhibit a wide range of fat globule sizes. As whey protein concentration increases, however, fibrous structures become apparent (Fig. 25A,B). The fibrous structures (possibly coagulated whey protein) are not seen at 1.5% whey protein and become visible at 3.0% whey protein. These structures may be responsible for not only the loss of cheese meltability but also the oiling-off defect noted in these cheeses during the meltability test.

Acid casein model process cheeses with added whey protein concentrate do not exhibit any abnormal physical structures. All acid casein cheeses are well-emulsified with uniform, small-sized fat globule spaces.

Figure 25. Scanning electron micrographs of rennet casein process cheese with added whey protein concentrate.

- A. 4.5% undenatured whey protein concentrate;
- B. 4.5% heat-denatured whey protein concentrate.



DISCUSSION

The meltability defect exhibited in the model process cheese systems cannot be attributed to any single constituent or process condition. Casein type, calcium concentration, whey protein concentration, type of emulsifying salt, and extent of pH conditioning of casein can all affect the meltability of process cheese. These causative agents and processes affected the meltability of rennet and acid casein model process cheeses in different ways.

Microstructure of rennet casein model process cheese was similar to regular process cheese (39). More complete emulsification resulted in poorer meltability in both process natural and rennet casein cheese. The microstructure of acid casein process cheese did not completely correlate with cheese meltability since some well emulsified cheese samples also melted well. The interactive effects of casein type, calcium concentration, emulsifying salt, and pH conditioning of the acid casein did not allow for complete predictability of cheese flow from the microstructure.

The present results can be used to further investigate the utilization of cheese base from ultrafiltration procedures in process cheese manufacture. The meltability defect noted when cheese base is used in process cheese

should not be attributed to any single causative agent or process.

The presence of whey proteins in cheese base makes the use of ultrafiltration technology attractive. The inclusion of these protein fractions in the cheese base and final product account for the cheese yield increase as well as nutritional enhancement. The whey proteins may inhibit meltability as indicated in this study but are not necessarily the major cause of a cheese melt defect. Cheese base prepared from UF whole milk retentate has approximately 4.0% (w/w) whey protein concentration. This interpolated level of whey protein in the model process cheeses still allowed for some cheese flow to occur.

The high level of calcium in cheese base compared to natural cheese used for processing may be a major cause of the melt defect. Emulsifying salts exhibit different degrees of effectiveness in sequestering calcium, particularly casein-bound calcium. Calcium levels exceeding that found in natural cheese may present more difficult and different emulsification problems in process cheese base manufacture.

Adjustment of milk pH levels prior to ultrafiltration can correct excess retained calcium in UF whole milk retentate and subsequently prepared cheese base. The pH adjustment-calcium level interaction may have to be correlated with the proper agent(s) so as to correctly emulsify the cheese system for adequate meltability.

CONCLUSIONS

1. Meltability of acid casein model process cheese increased as the conditioning pH of the casein increased. Maximum meltability was attained when 55 mL 5 N NaOH per 2 kg cheese was used to condition the acid casein.

2. Rennet casein model process cheese melted significantly better than any of the acid casein model process cheese at any pH conditioning level of the latter and when CIT was used as emulsifying salt.

3. Rennet casein model process cheese prepared with DSP or TSPP did not melt. Acid casein model process cheese prepared with DSP or TSPP (conditioned at pH 7.55 and 7.75, respectively) had excellent meltability.

4. Disodium oxalate added to rennet casein model process cheese prepared with DSP or TSPP enhanced meltability significantly.

5. Rennet and acid casein model process cheeses melted well when CIT or SALP were used as emulsifying salts. The rennet casein cheese melted slightly better than the corresponding acid casein cheese.

6. Inclusion of whey protein (undenatured or heat-denatured) into rennet or acid casein model process

cheese decreased meltability as the concentration of whey protein increased. Neither cheese model melted well at 4.5% (w/w) whey protein concentration.

7. Addition of whey protein (undenatured or heat-denatured) to rennet casein model process cheese caused loss of emulsion and oiling-off during the meltability test. Surface textural defects were apparent in this cheese when 3.0 and 4.5% whey protein was included.

8. Acid casein model process cheese increased in firmness and toughness as the level of pH conditioning increased. Toughness attained a maximum when 45 mL 5 N NaOH per 2 kg cheese was used.

9. Rennet casein model process cheese was less firm than acid casein model process cheese with the four emulsifying salts tested. In both model process cheeses TSPP produced the most firm while SALP the least firm cheese.

10. Rennet casein model process cheese was less tough than acid casein model process cheese when TSPP, CIT, or DSP are used as emulsifying salts. Acid casein cheese with SALP did not rupture under compression whereas rennet casein cheese with SALP produced the toughest cheese.

11. There were no significant correlations between meltability and firmness or toughness when whey protein was added to the model cheeses. Toughness decreased as whey

protein concentration increased and firmness was not clearly to whey protein concentration.

12. Rennet casein model process cheese emulsified with DSP became tougher when oxalate was added. Oxalate did not significantly alter firmness or toughness levels of rennet casein cheese emulsified with TSPP.

13. The degree of emulsification correlated negatively with meltability of rennet casein model process cheese. Extent of emulsification in acid casein model process cheese did not correlate with meltability in all cases.

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Appendix AComputer Program to Determine
Area Under Toughness Curve

```

100 DELETE X,Y
110 DIM X(200),Y(200)
120 WINDOW 0,100,0,100
130 VIEWSIZE 0,100,0,100
140 X=10
150 PAGE
160 FOR I=1 TO N
170 PRINT 0 12,45:1
180 CALL "WAIT",0.3
190 GIN 0 1:X(I),Y(I)
200 IF I<100 THEN 220
210 PRINT "E"
220 NEXT I
230 X(N+1)=X(I)
240 Y(N+1)=Y(I)
250 X(N+2)=X(I)
260 Y(N+2)=Y(I)
270 A=0
280 FOR I=1 TO N+1
290 A=A+(0.5*(Y(I-1)+Y(I))*(Y(I)-Y(I-1)))
300 NEXT I
310 PRINT 0 41:"AREA = ";
320 PRINT 0 41: USING 100.4X1(A/2)
330 INPUT ES
340 GO TO 150
350 IMAGE 78,20

```

APPENDIXES

Appendix A

Computer Program to Determine

Area Under Toughness Curve

```

100 DELETE X,Y
110 DIM X(200),Y(200)
120 WINDOW 0,100,0,100
130 VIEWPORT 0,100,0,100
140 N=125
150 PAGE
160 FOR I=1 TO N
170 PRINT @ 32,26:2
180 CALL "WAIT",0.3
190 GIN @ 1:X(I),Y(I)
200 IF I<100 THEN 220
210 PRINT "G"
220 NEXT I
230 X(N+1)=X(1)
240 Y(N+1)=Y(N)
250 X(N+2)=X(1)
260 Y(N+2)=Y(1)
270 A=0
280 FOR I=1 TO N+1
290 A=A+(X(I)+X(I+1))*(Y(I)-Y(I+1))
300 NEXT I
310 PRINT @ 41:"JAREA = ";
320 PRINT @ 41: USING 340:ABS(A/2)
330 INPUT E$
340 GO TO 150
350 IMAGE FD.2D

```

Appendix B

Treatment Description and Orthogonal
Contrast Coefficients Used in
Rheology Data Analysis

Description of Treatments

Treatment	Casein Type	Whey Protein Type	Amount of Whey Protein (%)
A	Acid	Undenatured	1.5
B	Acid	Denatured	1.5
C	Acid	Undenatured	3.0
D	Acid	Denatured	3.0
E	Acid	Undenatured	4.5
F	Acid	Denatured	4.5
G	Acid	None	0.0
H	Rennet	None	0.0
I	Rennet	Undenatured	1.5
J	Rennet	Denatured	1.5
K	Rennet	Undenatured	3.0
L	Rennet	Denatured	3.0
M	Rennet	Undenatured	4.5
N	Rennet	Denatured	4.5

Orthogonal Contrast Coefficients

	<u>CONTRAST</u>												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A	-1	0	-1	-1	+1	-1	+1	0	0	0	0	0	0
B	-1	0	-1	+1	-1	0	0	-1	+1	0	0	0	0
C	-1	0	-1	-1	+1	0	-2	0	0	0	0	0	0
D	-1	0	-1	+1	-1	0	0	0	-2	0	0	0	0
E	-1	0	-1	-1	+1	+1	+1	0	0	0	0	0	0
F	-1	0	-1	+1	-1	0	0	+1	+1	0	0	0	0
G	+6	-1	0	0	0	0	0	0	0	0	0	0	0
H	+6	+1	0	0	0	0	0	0	0	0	0	0	0
I	-1	0	+1	-1	-1	0	0	0	0	-1	+1	0	0
J	-1	0	+1	+1	+1	0	0	0	0	0	0	-1	+1
K	-1	0	+1	-1	-1	0	0	0	0	0	-2	0	0
L	-1	0	+1	+1	+1	0	0	0	0	0	0	0	-2
M	-1	0	+1	-1	-1	0	0	0	0	+1	+1	0	0
N	-1	0	+1	+1	+1	0	0	0	0	0	0	+1	+1

TREATMENTS

Appendix C

Statistical Tables

Table 15. Meltability¹ of model process cheese.

5 N NaOH (mL)	Casein Type	Melt Distance ² (mm)
-	Rennet	75.7 ± 5.9 ^a
25	Acid	32.5 ± 7.3 ^b
35	Acid	25.3 ± 3.8 ^c
45	Acid	49.2 ± 2.2 ^d
55	Acid	58.7 ± 6.8 ^e
65	Acid	58.8 ± 4.2 ^e
75	Acid	57.2 ± 8.0 ^e

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05.

Table 16. Analysis of variance of effect of casein treatment on meltability.

Source	df	SS	MS	F	Sig Level
Treatments	6	10625.90	1770.98	48.96	0.0001
Blocks	5	92.76	18.55	0.513	
Error	30	1085.24	36.17		
Total	41	11803.90			

Table 17. Meltability¹ of model process cheese with different emulsifying salts.

Sample		
Emulsifying Salt	Casein Type	Melt Distance ² (mm)
SALP	Acid	39.7 ± 4.1 ^d
DSP	Acid	80.3 ± 9.7 ^a
TSPP	Acid	66.2 ± 2.1 ^b
CIT	Acid	63.8 ± 8.8 ^b
SALP	Rennet	51.3 ± 2.2 ^c
DSP	Rennet	0.0 ± 0.0 ^e
TSPP	Rennet	4.8 ± 5.7 ^e
CIT	Rennet	71.3 ± 5.2 ^b

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05.

Table 18. Analysis of variance of the effect of emulsifying salts on meltability of model process cheese.

Source	df	SS	MS	F	Sig Level
Treatments	7	38479.48	5497.07	200.59	0.0001
Blocks	5	328.69	65.74	2.40	
Error	35	959.14	27.40		
Total	47	39767.31			

Table 19. Meltability¹ of model process cheese with added undenatured whey protein concentrate.

Sample		
Whey Protein (%)	Casein Type	Melt Distance ² (mm)
0.0	Acid	49.0 ± 4.0 ^{bc}
1.5	Acid	45.5 ± 4.0 ^c
3.0	Acid	23.7 ± 6.2 ^d
4.5	Acid	7.8 ± 6.5 ^e
0.0	Rennet	66.0 ± 3.7 ^a
1.5	Rennet	53.7 ± 4.1 ^b
3.0	Rennet	9.7 ± 2.5 ^e
4.5	Rennet	20.7 ± 7.4 ^d

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05.

Table 20. Analysis of variance of the effect of undenatured whey protein concentrate addition on meltability of model process cheese.

Source	df	SS	MS	F	Sig Level
Treatments	7	20076.31	2868.04	107.27	0.0001
Blocks	5	76.35	13.47	0.50	
Error	35	935.82	26.74		
Total	47	21079.48			

Table 21. Meltability¹ of model process cheese with added denatured whey protein concentrate.

Sample			Melt Distance ² (mm)	
Whey Protein (%)	Casein Type			
0.0	Acid	48.3 ±	6.6 ^{bc}	
1.5	Acid	54.0 ±	5.5 ^b	
3.0	Acid	37.7 ±	10.9 ^{cd}	
4.5	Acid	0.0 ±	0.0 ^e	
0.0	Rennet	68.5 ±	4.9 ^a	
1.5	Rennet	44.7 ±	14.4 ^c	
3.0	Rennet	28.0 ±	19.6 ^d	
4.5	Rennet	33.2 ±	5.9 ^{cd}	

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05

Table 22. Analysis of variance of the effect of denatured whey protein concentrate addition on meltability of model process cheese.

Source	df	SS	MS	F	Sig Level
Treatments	7	17349.58	2478.51	23.065	0.0001
Blocks	5	445.42	89.08	0.829	
Error	35	3760.92	107.46		
Total	47	21555.92			

Table 23. Meltability¹ of acid casein model process cheese with added undenatured and denatured whey protein concentrate.

Sample			Melt Distance ² (mm)	
Whey Protein (%)	WPC			
0.0	-		48.3 ±	6.6 ^a
1.5	Undenatured		50.3 ±	6.1 ^a
3.0	Undenatured		29.3 ±	11.2 ^b
4.5	Undenatured		5.5 ±	4.6 ^c
1.5	Denatured		54.0 ±	5.5 ^a
3.0	Denatured		37.7 ±	10.9 ^b
4.5	Denatured		0.0 ±	0.0 ^c

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05.

Table 24. Analysis of variance of the effect of undenatured and denatured whey protein concentrate addition on meltability of acid casein model process cheese.

Source	df	SS	MS	F	Sig Level
Treatments	6	17113.0	3852.17	51.74	0.0001
Blocks	5	230.98	46.20	0.83	
Error	30	1653.86	55.13		
Total	41	18997.83			

Table 25. Meltability¹ of rennet casein model process cheese with added undenatured and denatured whey protein concentrate.

Sample			Melt Distance ²	
Whey Protein (%)	WPC	(mm)		
0.0	-	68.5 ±	4.9 ^a	
1.5	Undenatured	56.2 ±	6.0 ^{ab}	
3.0	Undenatured	17.0 ±	11.1 ^d	
4.5	Undenatured	21.0 ±	7.2 ^d	
1.5	Denatured	44.7 ±	14.4 ^{bc}	
3.0	Denatured	28.0 ±	19.6 ^d	
4.5	Denatured	33.2 ±	5.9 ^{cd}	

¹ Mean ± standard deviation of triplicate determinations of duplicate samples.

² Values followed by same superscript not significantly different at P = .05.

Table 26. Analysis of variance of the effect of undenatured and denatured whey protein concentrate addition on meltability of rennet casein model process cheese.

Source	df	SS	MS	F	Sig Level
Treatments	6	12943.14	2157.19	17.31	0.0001
Blocks	5	340.50	68.10	0.55	
Error	30	3738.0	124.60		
Total	41	17021.64			

Table 27. Firmness¹ and toughness² of rennet and acid casein model process cheese.

5 N NaOH (mL)	Casein Type	Firmness ³ (N)	Toughness ³ (N-mm)
-	Rennet	3.21 ± 0.12 ^a	760 ± 50.2 ^a
25	Acid	3.42 ± 0.15 ^b	774 ± 30.0 ^a
35	Acid	3.77 ± 0.14 ^c	881 ± 126 ^{ab}
45	Acid	3.74 ± 0.19 ^d	1030 ± 165 ^b
55	Acid	4.08 ± 0.08 ^e	1020 ± 88.0 ^b
65	Acid	4.37 ± 0.14 ^f	991 ± 76.2 ^b
75	Acid	4.72 ± 0.18 ^g	1010 ± 187 ^b

- 1 Mean ± standard deviation of six measurements of duplicate samples.
- 2 Mean ± standard deviation of triplicate measurements of duplicate samples.
- 3 Values followed by same superscript not significantly different at P = .05.

Table 28. Analysis of variance of effect of casein treatment on firmness.

Source	df	SS	MS	F	Sig Level
Treatments	6	20.204	3.367	34.838	0.0001
Reps	7	0.6769	0.0967	4.429	0.001
Samples	28	0.6111	0.0218	2.262	0.01
Error	42	0.4052	0.0097		
Total	83	21.896			

Table 29. Analysis of variance of effect of casein treatment on toughness.

Source	df	SS	MS	F	Sig Level
Treatments	6	606413.92	101068.99	9.552	0.0001
Error	35	370316.51	10580.47		
Total	41	976730.43			

Table 30. Firmness¹ and toughness² of model process cheese with different emulsifying salts.

Sample			
Emulsifying Salt	Casein Type	Firmness ³ (N)	Toughness ³ (N-mm)
SALP	Acid	3.87 ± 0.41 ^a	0.0 ± 0.0 ^a
DSP	Acid	4.26 ± 0.32 ^b	1100 ± 83.5 ^b
TSPP	Acid	4.71 ± 0.18 ^c	1080 ± 101 ^b
CIT	Acid	4.50 ± 0.12 ^d	1120 ± 114 ^b
SALP	Rennet	3.19 ± 0.09 ^e	1050 ± 62.5 ^b
DSP	Rennet	3.47 ± 0.27 ^f	450 ± 91.4 ^c
TSPP	Rennet	3.99 ± 0.15 ^g	790 ± 81.5 ^d
CIT	Rennet	3.56 ± 0.09 ^h	960 ± 46.7 ^e

¹ Mean ± standard deviation of six determinations of duplicate samples.

² Mean ± standard deviation of triplicate determinations of duplicate samples.

³ Values with same superscript not significantly different at P = .05.

Table 31. Analysis of variance of effect of emulsifying salts on firmness of model process cheese.

Source	df	SS	MS	F	Sig Level
Salt	3	8.3123	2.7708	6.354	0.005
Casein	1	14.6641	14.6641	33.63	0.0001
Salt*Casein	3	0.2482	0.0827	0.19	
Rep(Salt*Casein)	8	3.4887	0.4361	16.90	0.0005
Sample(Salt*Casein*Rep)	32	0.8248	0.0258	3.909	0.05
Error	48	0.3176	0.0066		
Total	95	27.8557			

Table 32. Analysis of variance of effect of emulsifying salts on toughness of model process cheese.

Source	df	SS	MS	F	Sig Level
Salt	3	1513441.7	504480.56	43.21	0.0001
Casein	1	1520.7	1520.7	0.130	0.75
Salt*Casein	3	5164476.1	1721492.0	147.4	0.0001
Rep(Salt*Casein)	8	93411.2	11676.4	2.29	0.05
Error	32	163031.7	5094.7		
Total	47	6935881.4			

Table 33. Firmness¹ and toughness² of model process cheese with added undenatured and denatured whey protein concentrate.

Sample			Firmness ³ (N)	Toughness ³ (N-mm)
% Whey Protein	WPC	Casein Type		
0.0	-	Acid	4.46 ± 0.31 ^a	1160 ± 128 ^a
1.5	Undenatured	Acid	3.78 ± 0.19 ^b	1040 ± 53 ^b
3.0	Undenatured	Acid	3.76 ± 0.18 ^b	1020 ± 159 ^b
4.5	Undenatured	Acid	4.04 ± 0.48 ^c	657 ± 98 ^c
0.0	-	Rennet	3.62 ± 0.15 ^b	825 ± 79 ^{de}
1.5	Undenatured	Rennet	2.88 ± 0.20 ^d	538 ± 37 ^f
3.0	Undenatured	Rennet	2.85 ± 0.54 ^d	364 ± 44 ^g
4.5	Undenatured	Rennet	2.47 ± 0.22 ^e	278 ± 49 ^g
1.5	Denatured	Acid	4.38 ± 0.15 ^a	973 ± 123 ^b
3.0	Denatured	Acid	4.72 ± 0.69 ^f	879 ± 178 ^d
4.5	Denatured	Acid	5.38 ± 0.69 ^g	776 ± 48 ^e
1.5	Denatured	Rennet	3.38 ± 0.17 ^c	672 ± 32 ^c
3.0	Denatured	Rennet	3.31 ± 0.18 ^c	474 ± 44 ^f
4.5	Denatured	Rennet	3.14 ± 0.19 ^h	339 ± 25 ^g

- 1 Mean ± standard deviation of six measurements of duplicate samples.
- 2 Mean ± standard deviation of triplicate measurements of duplicate samples.
- 3 Values followed by same superscript not significantly different at P = .05.

VITA

Paul A. Savello

Candidate for the Degree of
Doctor of Philosophy

Dissertation: Meltability and Rheology of Model Process
Cheese Containing Acid and Rennet Casein

Major Field: Nutrition and Food Sciences

Biographical Information:

Personal Data: Born at Marlboro, Massachusetts,
September 25, 1944, son of Alexander and Erlleen
Savello; married Cheryl Adamson June 29, 1974;
children--Denise and Robert.

Education: Attended elementary school in Marlboro,
Massachusetts, graduated from Marlboro High School
in 1962, received the Bachelor of Science degree
from Bates College, Lewiston, Maine, with a major
in biology in 1966; 1979 completed the
requirements for the Master of Science degree at
Brigham Young University, Provo, Utah, with a
major in food science and nutrition; 1983
completed the requirements for the Doctor of
Philosophy degree at Utah State University, Logan,
Utah, with a major in nutrition and food sciences.

Professional Experience: 1966-1968, Peace Corps
Volunteer, Paysandu, Uruguay; 1968-1969, high
school science and math teacher, Jefferson High
School, Rochester, New York; 1969-1970, Peace
Corps Recruiter, Boston, Massachusetts; 1970-1971,
Peace Corps Volunteer Teacher of high school
science, Saipan, Mariana Islands, Trust Territory
of Pacific; 1972-1974, high school science
teacher, International High School of Caracas,
Venezuela; 1974-1975, laboratory assistant,
University of Utah Medical Center, Department of
Pediatric Neurology, Salt Lake City, Utah;
1975-1977, high school science teacher,
International High School of Caracas, Venezuela;

1977-1979, research and teaching assistant of Department of Food Science and Nutrition, Brigham Young University, Provo, Utah; 1979-1982, research assistant of Department of Nutrition and Food Sciences, Utah State University, Logan, Utah; 1982-1983, Research Scientist, Schreiber Foods, Inc., Green Bay, Wisconsin.

DEPARTMENT OF NUTRITION
& FOOD SCIENCES
Utah State University
750 North 1200 East
Logan Utah 84322-8700