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The Ohio State University

Ph.D. 1980

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A STUDY OF THE REHYDRATION PROPERTIES OF A MILK ANALOGUE CONTAINING SOY PRODUCTS AND CHEESE WHEY

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

VIRGINIA HARRIS HOLSINGER, B.S.

* * * * * *

The Ohio State University

1980

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ACKNOWLEDGEMENTS

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ABSTRACT

Rehydration properties of commercially manufactured samples of whey-soy drink mix, a spray dried milk analogue intended as a dietary supplement for preschool children in developing countries, were analyzed for physical quality attributes to determine the factors which control the physical stability of the rehydrated product.

Particle structure of intact powders by light microscopy (LM) and scanning electron microscopy (SEM) showed slightly collapsed spherical particles with smooth surface structure and some aggregation. Martin's diameters of individual particles ranged from three to 174 micrometers with the largest number in the 5 - 35 micrometer range. After reconstitution with water to 15% total solids, samples prepared with full fat flour contained many irregular particles with convoluted surfaces that appeared fibrous in nature. Reconstituted samples prepared with toasted defatted flour contained more nearly spherical particles with rough-textured surfaces which appeared to be aggregates of small particles. When the rehydrated particles were differentially stained with acridine orange and phosphine dyes, ultraviolet microscopic examination revealed many of them to be proteinaceous material coated with fat droplets.

Response surface methodology was used to evaluate optimal levels of three processing variables, homogenization pressure (HP), total
solids of the homogenized mixture (TS) and emulsifier level on disperability, NSI, free fat content, $d_e$ and phase separation after reconstitution. Conditions studied were HP of 1800-500, 2500-500, 3000-500 psi (126.6-35.2, 175.8-35.2, 211.0-35.2 kg/cm$^2$) emulsifier levels of 0 and 0.5% and TS of 13.7%, 26% and 40%.

Emulsifier effects were shown to be undesirable. Emulsifier destabilized the fat emulsion of the whey-soy system during the drying process, resulting in reduced disperability and increased free fat content of the powder and increased phase separation and decreased NSI after reconstitution.

The size parameter, $d_e$, was not significantly affected by the processing variables, other than the presence of emulsifier, and was not the controlling factor in phase separation; both phase separation and $d_e$ were related to viscosity which was significantly influenced by all three processing variables. Phase separation was least in samples homogenized at high pressure and at high total solids; these samples had the highest viscosities upon reconstitution.

All results suggested that formation of a fat-protein complex during homogenization was responsible for reduced settling; complex formation was inhibited by emulsifier. Bonding was hydrophobic or electrostatic in nature; input of mechanical energy into the system was sufficient to release the fat from the particulates.

NSI was significantly affected by all processing variables. An increase in NSI caused by homogenization was maintained after drying. Solubility studies with homogenization in the presence of chemical additives suggested that soybean protein conformation was altered due
to breaking hydrophobic and disulfide bonds. Ultracentrifuge analysis showed that the increase in NSI brought about by homogenization was due to resolubilization of 7S and 11S globulin fractions of the soybean flour proteins.
INTRODUCTION

Burgeoning world population growth has resulted in serious inequalities in the available food supply. Malnutrition is widespread; the most critical nutrition problem is protein-calorie malnutrition of young children, pregnant and lactating women, particularly in the so-called developing nations in which two-thirds of the world's population live (1). The basic cause is a diet which is likely to be low in protein, provide insufficient energy and often be marginal in other nutrients.

High quality animal proteins are scarce in many of the developing nations where food shortages are most acute. Therefore, plant proteins, particularly oilseed proteins, have become the most attractive alternative sources of low-cost protein foods. Among the oilseeds, soybean protein is the most promising because it contains high levels of essential amino acids and manufacturing techniques for the production of a wide variety of food products are already well-developed (2).

Milk production is almost always inadequate to meet the nutritional needs of the population in developing countries. For many years, commodities and processed foods, including nonfat dry milk, have been purchased by the U.S. Department of Agriculture (USDA) for distribution to needy people abroad by the U.S. Agency for International Development (AID) through the United States Voluntary
Agencies, through government-to-government bilateral agreements, and through contributions to the World Food Program of the Food and Agricultural Organization of the United States (3). Over 100 million people in about 90 developing countries have participated in these programs (4).

In 1973, supplies of nonfat dry milk suddenly became short and priced out of reach for overseas distribution by the U.S. Food-for-Peace child feeding programs (5). Therefore, a joint USDA-AID effort was mobilized to develop a nutritious beverage powder designed as a dietary supplement for preschool children receiving inadequate protein but not intended as the sole source of food. The result was whey-soy drink mix, a high protein, high fat, high calorie product with a protein-to-fat balance similar to milk (6). The product contained sweet cheese whey solids, soybean flour, soybean oil, corn syrup solids and added vitamins and minerals. 13.3 million kilograms have been shipped overseas since 1974 (7).

Since specifications for WSDM were first drawn up (8), research has continued in an effort to improve the product further. A major unsolved problem is the fact that unlike nonfat dry milk, WSDM shows some degree of settling and phase separation on standing, after reconstitution to 15% total solids, for beverage use. Although no complaints have been received from abroad, AID officials view this problem with concern.

The overall objective of this work was to study the rehydration properties of WSDM. The first approach was to evaluate properties related to reconstitutability and suspension stability of commercially
prepared samples manufactured under specifications. This was investigated by measuring such properties as dispersibility, sinkability, free fat content, nitrogen solubility index, particle size distribution and degree of settling after reconstitution. Particle structure before and after rehydration was also investigated by light and scanning electron microscopy. The second approach was to examine the effects of three processing variables, homogenization pressure, total solids of the homogenized mixture and inclusion of emulsifier in the formulation on rehydration characteristics. This was accomplished by computing the response surfaces in order to select combinations of variables to optimize rehydration properties and suspension stability. The third approach was to investigate factors influencing the dispersibility of soybean proteins in cheese whey. Parameters such as pH of the whey, calcium ion concentration and treatment with disulfide bond reducing agents were evaluated for their effects on solubility of the proteins in soybean flour.
REVIEW OF LITERATURE

I. Soybeans

A. Historical

Soybean has been a traditional food crop in Southeast Asia since before written history. However, significant commercial soybean production in the United States only began in the early 1920's (9). Since then, average yields and total production have increased sharply; from a crop of less than 109,000 metric tons in 1922, U.S. production in 1977 came to 49.6 million metric tons, representing 19.8 million metric tons of protein (10). The U.S. produces about 2/3 of the world's supply.

B. Soybean Seed Composition and Ultrastructure

Soybeans are typical legume seeds that differ in color, size and shape depending on the variety. Commercial soybeans are composed of about 8% hull, 90% cotyledon and 2% hypocotyl and plumule. The proximate analyses of whole soybeans and seed parts are given in Table 1 (11). The major constituents, protein and oil, comprise about 60% of the bean; about 1/3 consists of carbohydrate, including polysaccharides, stachyose (3.8%), raffinose (1.1%) and sucrose (5.0%). Phosphatides, sterols, ash and other minor constituents are also present (9).

The internal seed structure was described by Wolf (12). The hull consists of an epidermis of palisade cells, large (30-70 micrometers) hourglass cells, 6 to 8 layers of spongy parenchyma cells, aleurone cells and compressed endosperm cells. The cotyledon is
Table 1. Average Composition of Soybeans and Seed Parts of 6 U.S. Varieties

<table>
<thead>
<tr>
<th></th>
<th>Whole Soybeans</th>
<th>Full Fat Cotyledons</th>
<th>Full Fat Hypocotyl</th>
<th>Hull</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>40.4</td>
<td>43.4</td>
<td>40.8</td>
<td>9.0</td>
</tr>
<tr>
<td>Fat</td>
<td>22.3</td>
<td>24.3</td>
<td>12.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Ash</td>
<td>4.9</td>
<td>5.0</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Nitrogen free extract plus fiber</td>
<td>31.9</td>
<td>27.4</td>
<td>42.7</td>
<td>86.2</td>
</tr>
</tbody>
</table>

Kawamura (11)
covered with an epidermis of small cubical cells followed by a layer of larger cells. The remainder of the cotyledon is composed of polygonal cells 30-50 micrometers by 15-25 micrometers. These cells contain numerous subcellular inclusions that are the storage sites for proteins and lipids. The protein bodies (aleurone grains) are nearly spherical and 2-20 micrometers in diameter; oil is contained within small particles called spherosomes (0.2-0.5 micrometers in diameter) interspersed among the protein bodies.

Three procedures have been developed to isolate and characterize the soybean protein bodies (13, 14, 15). All methods used density differences to effect separation.

Tombs (14) isolated the purest fraction of protein bodies by homogenizing 350 mesh defatted flour in 20% sucrose - pH 5.0 citrate buffer and centrifuging in a 70-90% sucrose gradient. Tombs reported that defatting was necessary because homogenization of the intact cotyledon was difficult and intractable emulsions of the protein bodies and spherosomes formed.

Tombs isolated two protein body fractions, a light fraction with density less than 1.30 and a heavy fraction with density 1.30-1.32. The light fraction contained 97.5% protein (N x 5.8); the heavy fraction was almost 80% protein but appeared to contain contaminants such as cell wall fragments. Tombs found only 11S protein in the protein bodies by electrophoresis. All other proteins, including trypsin inhibitor, were presumed to be of cytoplasmic origin. However, both Wolf (16) and Catsimpoolas et al (17) found that the proteins of protein bodies are heterogeneous. Wolf showed by
ultracentrifuge that 2S, 7S, 11S and 15S components similar to those found in the water extracted proteins of defatted soy meal were present, except that 2S was in lower concentration in the protein bodies. Catsimpoolas et al (17) did not confirm the presence of 2S in the storage protein. These results suggested that trypsin inhibitor was either not present or in low concentration in the protein bodies.

Tombs (14) observed numerous free, apparently intact protein bodies in the 350 mesh flour. Saio and Watanabe (13) reported that protein bodies burst when water-soaked beans were steamed at 115°C for 30 min. Tombs (14) also observed that protein bodies swelled, often doubling in size when 325 mesh soy meal was suspended in water. Eventually they ruptured, releasing small granules less than 0.5 micrometer in diameter. This effect was prevented if the suspension was made in an aqueous medium buffered to pH 5, the isoelectric point of 11S protein.

Wolf (16) and Wolf and Baker (18), employing scanning electron microscopy, noted protein bodies 1 to 10 micrometers in diameter in dry milled flour. However, after density fractionation by Tombs' procedure, the largest protein bodies observed were 3 micrometers in diameter. Wolf suggested that the protein bodies are of two types: those 1 to 3 micrometers in diameter that are stable during isolation by sucrose density gradient centrifugation and those of larger diameter that are not stable. Breakdown of the protein bodies also appeared more extensive in freeze-dried samples than in vacuum dried samples.
Wolf and Baker (19) used SEM to examine the structure of freeze-fractured cotyledons. Because the cell wall apparently did not rupture when wetted, they concluded that mechanical rupture is necessary for efficient protein recovery during soymilk production.

Wolf and Baker (19) also examined full fat and defatted flours in this study. In full fat flour, they observed numerous spherical or elliptical particles 6-10 micrometers in diameter that apparently were protein bodies that remained intact during commercial processing.

Upon examination of defatted flours that had received increasingly severe heat treatments, the white flour (NSI 60-70) showed numerous intact protein bodies whereas the fully toasted flour (NSI 15-25) contained fewer particles identifiable as protein bodies. Some new structural elements also seemed to form during further processing. When isoelectric isolates were spray dried as insoluble dispersions, rough particles resulted; whereas when an isolate in the neutralized proteinate form was spray-dried, the soluble protein formed a smooth continuous film during water evaporation yielding smooth, partially hollow particles that appeared as spheres or partially collapsed spheres.

Under extremely high magnification (ca. 20,000x), Bair and Snyder (20) observed by SEM that the protein bodies of full fat flour appeared very smooth and little affected by processing. However, the protein bodies of defatted toasted flour were distorted and rough and appeared to be agglomerates of very small particles on the order of 0.1 micrometer in diameter. The authors had no explanation for these differences. However, the findings might explain the granules <.5μ
in diameter observed by Tombs (14) when water swollen protein bodies ruptured. The protein bodies observed in full fat flour may really
be composed of closely packed subunits held together by a combination
of ionic, covalent, hydrogen and hydrophobic bonds in a fashion
similar to the subunits of the casein micelles. Lipid may also be
dispersed through the interstices between subunits. Solvent treatment
to extract the lipid could disrupt the structure, roughening the
appearance and making the interior of the particle more readily
accessible to and wetted by water; the resultant swelling could
cause mechanical rupture of the unit. Since this effect does not
occur in a buffered solution, polar effects might be most important
in holding the unit together.

C. Soybean Proteins

Soybeans contain protein as the major constituent. Average
proximate composition is: protein 40%, oil 21%, carbohydrate 34%,
and ash 5% (9). When a 60 pound bushel of soybeans is processed by
hexane extraction, typically 43.3 pounds of defatted meal and 11
pounds of oil are obtained (21). The defatted meal or flour has a
protein content of about 50%.

The average consumption of soybean protein in the U.S. is
still small, about 2.5 g/per capita per day (22). Nevertheless,
there are individuals within the U.S. population that are consuming
appreciably more than average levels because soy proteins are being
used as meat extenders and analogues in institutional feeding programs
such as schools and nursing homes. Therefore, the nutritional
properties of soybean proteins are assuming increasing importance.
1. Nutritional properties of soybean proteins

The traditional approach for determination of soybean protein quality has been to feed it to experimental animals, usually growing rats and determining the protein efficiency ratio (PER). The PER value is the weight gain divided by the weight of protein consumed under standardized conditions (23). Results of such tests have consistently shown soybean protein to have a lower PER than casein or egg protein (22). Protein quality is dependent upon the relative proportions of essential amino acids. Table 2 (24, 25, 26) lists the essential amino acid content of the proteins of defatted soybean meal along with that of egg protein. The revised FAO/WHO reference protein pattern for man is shown for comparison. The histidine content is shown because it is required for infants and its dispensability for adults has been questioned (27).

Growing rats require higher levels of both lysine and methionine + cystine than humans do and, in addition, require a dietary source of arginine whereas humans do not (28). In the case of soybean protein, methionine + cystine is limiting for the rat; this could account for the consistently lower PER's observed.

a. Nutrition studies with humans

The ultimate answer to soybean protein quality must come from studies with humans. Young et al (29) and Wolf (22) have both extensively reviewed studies of soybean protein quality measurements with infants, young children and adults (only the most important studies will be mentioned here).
Table 2. Essential Amino Acids of Soybean Protein Compared to Egg Protein and the FAO Reference Pattern

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>FAO/WHO reference (^1)</th>
<th>Defatted soybean meal (^2)</th>
<th>Egg Protein (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>5.5</td>
<td>6.9</td>
<td>6.6</td>
</tr>
<tr>
<td>His</td>
<td>-</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Thr</td>
<td>4.0</td>
<td>4.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Cys + Met</td>
<td>3.5</td>
<td>3.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Val</td>
<td>5.0</td>
<td>5.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Ileu</td>
<td>4.0</td>
<td>5.1</td>
<td>6.6</td>
</tr>
<tr>
<td>Leu</td>
<td>7.0</td>
<td>7.7</td>
<td>9.1</td>
</tr>
<tr>
<td>Tyr + Phe</td>
<td>6.0</td>
<td>8.9</td>
<td>10.1</td>
</tr>
<tr>
<td>Trp</td>
<td>1.0</td>
<td>1.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(^1\)FAO (24)

\(^2\)Rackis, et al (25)

\(^3\)Orr & Watt (26)
Foman and coworkers (30) have carried out many studies with infants in which soy protein isolate fortified with DL- or L-methionine was compared to cows' milk. Examination of the nitrogen balance showed the fortified soy protein isolate to be equal to bovine milk protein in promoting nitrogen retention. Linear growth and/or body weight gain of infants receiving soy equalled that of those receiving cows' milk. In another study (30), a direct comparison of growth was made on infants fed cows' milk, human milk and methionine-fortified-soy protein isolate; there were no statistically significant differences in growth among infants receiving protein from the three different sources. When soy protein isolate was fed with and without methionine supplementation, food intake and growth showed no differences but serum urea was lower in infants fed the methionine supplement; these results suggested that protein quality was improved by the added methionine but this was not confirmed by nitrogen retention measurements. Torun and coworkers (31) conducted tests with children 19 to 44 months old; two soybean protein isolates were compared to cows' milk as reference. Nitrogen balance studies showed that the isolates yielded nitrogen retentions similar to milk; digestibilities were also comparable. These tests were conducted without methionine supplementation; results were contrary to what would be expected from rat bioassays.

Zezulka and Calloway (32) determined the minimum intake of soy protein isolate with and without added methionine required to meet the amino acid requirements of adult men. Their results
showed that 3 g of soy protein nitrogen gave a negative nitrogen balance but when the isolate was fortified with methionine to bring the total sulfur amino acid to the FAO/WHO (24) recommended 900 mg/day, the men were in balance. At intakes of 6.0 g of soy protein nitrogen or higher, the men were in positive nitrogen balance without added methionine. The results showed that methionine was limiting at low intake levels but at daily soy protein intakes of 38 g or higher, the sulfur amino acid requirements were met. Young et al (29) also examined the effect of methionine supplementation on nitrogen balance when isolates were fed at a level of 0.51 g protein/kg weight/day. At this low level, 1.1% methionine supplementation gave a positive response equal to that obtained with egg protein. If the level of isolate was increased to 0.8 g/kg weight/day to meet the dietary allowance for total protein, added methionine had no effect.

On the basis of these studies, Young et al (29) concluded that for young children and adults, under normal conditions of soy protein intake, methionine supplementation of good quality products is unnecessary and possibly undesirable. For neonate feeding, however, supplementation of soy-based formulas with methionine may be beneficial; however, the amount to be added is considerably less than that obtained from rat bioassays.

b. Antinutritional factors

Adequate processing of soybeans before consumption must be carried out because of the presence of biologically active factors that can affect nutritional quality. On the other hand, some
processing techniques can also bring about deleterious nutritional effects.

1. Trypsin inhibitors

Among the biologically active factors present in the soybean, only the protease inhibitors have been shown to have significant antinutritional effects on animals consuming diets containing soybean protein (33). Kunitz (34) isolated a protein from raw soybeans that was uniquely capable of combining with trypsin to form an inactive complex. It was originally thought the trypsin inhibitor was responsible for the growth depression observed in animals fed raw soybeans because of its ability to inhibit intestinal proteolysis. Supplementation with methionine improved the nutritive value of raw soybeans, suggesting that the trypsin inhibitor somehow interfered with the availability of methionine from the raw bean (35). However, it appears that the true explanation regarding the mode of action of trypsin inhibitor in the intact animal is more complicated (33). There is little doubt that hypertrophy of the pancreas is the primary physiological effect produced by feeding raw soybeans or the isolated inhibitor (36). Hypertrophy of the pancreas is accompanied by an increase in secretory activity, suggesting that the growth depression caused by the trypsin inhibitor might be the result of an endogenous loss of essential amino acids secreted by a hyperactive pancreas (37). Since pancreatic enzymes such as trypsin are very rich in the sulfur amino acids, pancreatic hypertrophy drains the body of these amino acids to meet the increased need for enzyme synthesis. This loss in sulfur containing amino acids accentuates
the situation with respect to soybean protein, already deficient in these amino acids.

Trypsin inhibitor is readily inactivated by heat (38). Steaming for 10 minutes inactivates about 80% of the trypsin inhibitor activity and the PER reaches a maximum (22). Only about 50% inactivation of the inhibitor is necessary to eliminate pancreatic enlargement (39).

Because trypsin inhibitors affect the pancreas in animals, there is concern about their possible effects when ingested by humans. Although moist heat will reduce inhibitor activity to low levels in soybean flours and grits, not all soybean products are processed this way. Toasted and textured flours have about 10% of the inhibitor activity found in raw soy flour; some concentrates and isolates have inhibitor levels ranging as high as 20-30% of the value found in the raw flour (22).

Preliminary long term studies with rats have revealed no problems caused by residual trypsin activity (40). In addition, there is some question of whether the soybean inhibitors actually inhibit human trypsin. Human trypsin exists in 2 forms, a cationic species, the major component, and an anionic species that is responsible for less than 1/3 of the total trypsin activity of pancreatic juice. While the anionic species is stoichiometrically inhibited by soybean trypsin inhibitor, the cationic species is only very weakly inhibited (41). There also seems to be a direct relationship between pancreatic size and sensitivity of pancreatic response to the inhibitor (33). Pancreases of those animal species whose
weights exceed 0.3% of the body weight became hypertrophic, whereas those animals whose pancreas weights fell below this value showed no hypertrophic effects. These results suggest that the human pancreas would be unaffected by soybean trypsin inhibitor, but there is no direct experimental evidence to prove this.

2. Lectins

In addition to trypsin inhibitors, soybeans contain several lectins, comprising 1-3% of the protein of defatted soybean flour (42). Lectins are the so-called phytohemagglutinins which bind to the sugar components of a wide variety of compounds. With red blood cells, the interaction of lectins with glycoproteins results in in vitro agglutination. Soybean lectin is also readily destroyed by heat. However, Turner and Liener (43) clearly showed that soybean lectin is minor in contributing to the poor nutritive value of raw soybeans, because PER was essentially unchanged by lectin removal, but considerably improved upon subsequent heat treatment.

3. Lysinoalanine

A number of investigators have demonstrated that alkali treatment of proteins can cause chemical changes that result in the formation of new amino acids such as ornithinoalanine (44), lanthionine (45) and lysinoalanine (46, 47). Lysinoalanine formation is believed to proceed from dehydroalanine and lysine by way of $\beta$-elimination from amino acids such as cystine and serine with functional groups at the $\beta$-carbon atom (48). Lysinoalanine was shown to have a nephrotoxic effect in rats fed alkali treated soy
proteins (49, 50). deGroot and Slump (51) could not reproduce the lesion under the conditions described originally by Woodard and Alvarez (49). O’Donovan (52) has pointed out that LAL fed to animals other than rats did not cause the renal cytomegalic lesion and suggested that the LAL toxic effect was species specific. Free LAL is 10 times more active than peptide linked LAL in alkali treated proteins in inducing the lesion (53). LAL has also been reported in foods other than soybean proteins (54).

4. Maillard reaction

Heat treatment is necessary to improve the nutritive value of soybeans (55). In general, the amount of improvement in nutritional value is dependent on temperature, time of heating and moisture conditions. Excessive heat treatment will damage the protein and impair nutritional value. Particularly vulnerable is lysine, which, if not destroyed by overheating the soy protein, may be rendered nutritionally unavailable (56, 57). The unavailability is due to the fact that the ε-amino groups of lysine interact with reducing sugar carbonyls in what is known as the browning or Maillard reaction (58). In the case of soybeans, the hydrolysis of sucrose, a natural constituent, during heat treatment, can give rise to reducing sugars that will interact with lysine (9).

Excessive heat during processing may also destroy as much as 2/3 of the cystine content (56). As the sulfur amino acids are already limiting in soybean proteins, excessive heat would only accentuate this problem.
Arginine, tryptophan, histidine and serine are also partially destroyed or inactivated by excessive heat treatment (55). Because these amino acids are not limiting in soybean protein, their partial loss does not affect the nutritive value.

2. Chemical and physical properties of soybean proteins

Much of the subsequent behavior of soybean proteins especially when incorporated as an ingredient in fabricated foods may be attributed to their chemical and physical properties. The following discussion will emphasize the complexity of these materials and suggest that they can undergo a wide range of reactions in food systems.

a. Molecular size distributions

Undenatured soybean proteins consist of discrete groups of proteins with molecular weights ranging from 8000 to 600,000. Ultracentrifuge patterns have provided the basis for the popular classification of the water soluble protein fractions. Naismith (59) introduced a nomenclature system whereby the fractions produced by ultracentrifugation of an aqueous dispersion were designated 2S, 7S, 11S and 15S where S stands for the sedimentation coefficient in Svedbergs. Approximate amounts of each sedimenting fraction are given in Table 3 (60). Physical properties of some purified soybean proteins are given in Table 4. The 7S and 11S fractions comprise more than 60% of the total protein, and about 80% of the proteins have molecular weights of 100,000 or more.

The 2S fraction contains the low molecular weight proteins: the trypsin inhibitors, 2 globulins, allantoinase and
Table 3. Approximate Amounts and Components of UC Fractions of Water Extractable Soybean Proteins

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of Total</th>
<th>Components</th>
<th>Molecular Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>2S</td>
<td>22</td>
<td>Trypsin inhibitors</td>
<td>8000, 21,500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytochrome c</td>
<td>12,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3S globulin</td>
<td>18,200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8S globulin</td>
<td>32,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allantoinase</td>
<td>50,000</td>
</tr>
<tr>
<td>7S</td>
<td>37</td>
<td>Hemagglutinins</td>
<td>110,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoxygenases</td>
<td>108,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 amylase</td>
<td>61,700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7S globulin</td>
<td>186,000-210,000</td>
</tr>
<tr>
<td>11S</td>
<td>31</td>
<td>11S globulin</td>
<td>350,000</td>
</tr>
<tr>
<td>15S</td>
<td>11</td>
<td>~</td>
<td>~600,000</td>
</tr>
</tbody>
</table>

Wolf (60)
Table 4. Physical Properties of Purified Soybean Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>$S_{20,w} \times 10^{13}$</th>
<th>MW</th>
<th>I.P.</th>
<th>$\eta_{1\text{ cm}}^{280\text{ mu}}$</th>
<th>Di/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowman-Birk Trypsin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitor</td>
<td>2.3</td>
<td>24,000</td>
<td>4.2</td>
<td>4.8</td>
<td>--</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>1.8</td>
<td>12,000</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Kunitz Trypsin Inhibitor</td>
<td>2.29</td>
<td>21,500</td>
<td>4.5</td>
<td>9.44</td>
<td>0.04c</td>
</tr>
<tr>
<td>Hemagglutinin</td>
<td>6.4</td>
<td>105,000</td>
<td>6.1</td>
<td>15.7</td>
<td>--</td>
</tr>
<tr>
<td>Lipoxygenase</td>
<td>5.62</td>
<td>102,000</td>
<td>5.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7S globulin</td>
<td>7.92</td>
<td>186,000</td>
<td>4.9</td>
<td>5.47</td>
<td>0.0638</td>
</tr>
<tr>
<td>11S globulin</td>
<td>12.2</td>
<td>333,000</td>
<td>5.0</td>
<td>9.2</td>
<td>--</td>
</tr>
</tbody>
</table>

Smith and Circle (9)
and cytochrome c.

The 7S fraction contains more than 1/3 of the total protein but at least 4 different proteins are present: β-amylase, lipoxygenases, hemagglutinins and 7S globulin. The first three are most likely minor constituents (60). Gel filtration separated the 7S fraction into 4 subfractions (61); gel electrophoresis showed three bands in a 7S fraction isolated by sucrose density gradient centrifugation (62).

About one-third of the total protein is found in the 11S fraction. Only one protein, the 11S globulin, glycine, has been found in this fraction (60). The 15S fraction makes up the remaining 1/10 of the protein; it may be a polymer of the 11S globulin (60).

Complexity of the proteins has been further demonstrated by chromatography on hydroxyapatite (63), starch gel electrophoresis (64) and immunoelectrophoresis (65, 66, 67).

b. Properties of 7S and 11S globulins

Soy proteins are biomacromolecules with numerous possibilities for chemical bonding: hydrophilic, hydrophobic, hydrogen, covalent and ionic. The availability of the groups available for reaction is governed by conformational arrangement of the molecule which controls the exposure or burial of the reactive sites. Factors affecting protein conformation are: 1. intrinsic, such as the size and shape of the molecule, amino acid sequence and polarity of the reactive groups, the planar structure of the peptide bond and the number of covalent disulphide crosslinkages, and 2. environmental, such as the temperature, pH, ionic strength and type of solvent.
The bulk of the soy proteins are globular which means that their polypeptide chains are compactly folded with the hydrophobic groups shielded on the inside; the surface polar groups are dehydrated. Globular proteins may also contain widely varying segments in α-helix or β-conformation depending on the amino acid sequence.

1. Primary structure

Little is known about the amino acid sequence of the soybean proteins; only the N-terminal amino acids of the 7S globulins are known.

2. Quaternary structure

Both the 7S and 11S globulins are made up of smaller subunits that interact very specifically to form the parent globulins.

The 7S globulin contains 9 amino terminal residues, indicating that the molecule contains at least 9 polypeptide chains (68). In acid solution at low salt concentrations, the 7S protein forms 2 species, a 2S and a 5S. Conversion of the 7S protein into the slower sedimenting forms is inhibited by salts and may be reversed by dialyzing to pH 7.6, 0.5 ionic strength (69).

The 7S globulin structure may be dissociated by reagents such as 8M urea or 4M guanidine hydrochloride; the molecular weight has been shown to decrease from 180,000 for the native structure to 22,500 to 24,000 (70).

At pH 7.6, 0.5 ionic strength, the 11S globulin has a quaternary structure made up of 12 subunits (71). However,
only 6 subunits separated on isoelectric focusing in urea-mercapto-ethanol. Catsimpoolis (72) has proposed a dimer of 2 identical "monomers," each containing 6 subunits. The quaternary structure of the 11S globulin molecule is disrupted by high and low pH, high concentrations of urea, detergents, low ionic strength and temperatures above 80°C. (73).

The spinning of fibers from protein isolates is a good example of a food system in which the quaternary structures of the 7S and 11S globulins are disrupted during processing. When dissolved in strong alkali in the first step of the spinning process, the structure breaks down into subunits and unfolds; upon coagulation in the acid bath after pumping through the spinnerettes, the fibers are only slightly soluble in solvents that dissolve the original protein. Apparently the unfolded subunits interact non-specifically instead of refolding and reassembling into the quaternary structure found in the native proteins (74).

3. Secondary and tertiary structure

Fukushima (75) examined the secondary and tertiary structures of a 7S globulin; optical rotatory dispersion and IR measurements did not detect appreciable α-helix but suggested that antiparallel β-structure and disordered regions predominated. Fukushima concluded that the proteins are folded compactly even though large regions of non-helical structure occurred because only about 40% of the peptide hydrogens exchanged with deuterium and UV difference spectra in urea showed that tyrosine residues were buried in the interior while tryptophan residues were accessible to solvent.
Fukushima proposed that hydrophobic bonding is important in maintaining the tertiary structure.

Fukushima's work also showed that the 11S globulin had secondary and tertiary structures similar to those of the 7S globulin. The 11S molecule therefore also appears to be a compact structure stabilized by hydrophobic bonds.

4. Disulfide polymerization

When the water extracted proteins are ultracentrifuged in the presence of 0.01M mercaptoethanol, the amount of 7S and 11S proteins increases while the fast sedimenting material disappears (76). The fast sedimenting materials are apparently disulfide polymers of 7S and 11S proteins. Since the polymers were found in extracts that received a minimum of treatment, they probably pre-existed in the defatted meal (9).

Insolubilization of the 7S and 11S proteins as a result of disulfide polymerization occurs during isoelectric precipitation of the globulins (77) during preparation of soy protein isolates. Disulfide polymers cause turbidity (78) and increase viscosity (79) of soybean protein solutions.

c. Effects of heat on soybean proteins

The term "denaturation" is always introduced when discussing the effects of heat on proteins. Neurath's (80) definition of denaturation is widely used, that is, "any non-proteolytic modification of the unique structure of a native protein, giving rise to definite changes in chemical, physical or biological properties."

Denaturation of the soybean proteins by moist heat is well known and
heat has traditionally been used to eliminate the antinutritional factors in soybean meals and flours (81). Even though heat treatment is commonly employed in processing soybeans, the physical and chemical changes occurring in the proteins at the molecular level are still poorly understood (60).

Early studies were concerned with the effects of heat and moisture on defatted meal and concentrated on the amount of soluble nitrogen remaining after heating (82). Shibasaki et al (83) reported changes in extractability of the different protein components as a function of heating time. About 3/4 of the proteins in steamed soybean meal were soluble in pH 8.6 buffer containing 0.1M mercaptoethanol plus 8M urea whereas mercaptoethanol in buffer dissolved only about 1/4 of the denatured protein.

Disulfide bonds are broken by mercaptoethanol; since hydrogen and hydrophobic bonds are believed to be broken by 8M urea, these bonds are likely to be major factors causing insolubility of heated meal proteins (60). Fukushima (84) showed that when soybean meal was autoclaved above 100°C with an equal weight of water, the soluble protein decreased to a minimum and then increased again as heating continued. Heating at 100°C with greater amounts of water solubilized large amounts of protein even after heating for 30 minutes.

Mann and Briggs (85) found that heating aqueous extracts of defatted meal precipitated whey or non-globulin (by electrophoresis) proteins. Heating the extract in buffer at pH 7.6 and 0.1 ionic strength prevented precipitation; electrophoresis showed a
single nearly symmetrical peak, apparently an aggregate formed by interactions among the different components. When l1S globulin was added to the whey proteins it was also incorporated into the aggregate.

Watanabe and Nakayama (86) confirmed the formation of aggregates at pH 7.0. However, after heating for 10 minutes at 100°C, only a 2S and a 5S fraction were detected by ultracentrifuge; after 30 minutes heating, the 5S fraction disappeared, leaving only the 2S fraction. The combination of heat and pressure applied in the extrusion process also resulted in a breakdown of 15S, l1S and 7S proteins (87).

Only limited data are available on heat denaturation of the purified proteins. Kunitz (88) carried out a classical study of the kinetics and thermodynamics of heat denaturation of crystalline trypsin inhibitor. This protein is inactivated when heated in solution but regains its activity reversibly when cooled. Side reactions must occur when soybean meal is heated, however, because there are no reports of reversible trypsin inhibitor activity in heated meal (9).

Catsimpoolis et al (89) followed changes in l1S globulin during heating by using turbidity measurements. Turbidity increased rapidly over 70°C and the protein precipitated at 90°C. Disc electrophoresis and immunodiffusion both showed that although dissociation into subunits occurred at 90°C, undissociated l1S globulin also remained. Wolf and Tamura (90) followed the changes in heated l1S globulin as a function of time with the ultracentrifuge. After 5 minutes at 100°C, l1S component disappeared and was converted
into a soluble aggregate and a 3-4S fraction. After further heating, the aggregate precipitated leaving only the 3-4S component in solution. Heating in 0.01M mercaptoethanol hastened the precipitation; precipitation still occurred in 0.5M mercaptoethanol, showing that sulfhydryl-disulfide interchange is not likely to contribute to the precipitation reaction. When the protein was heated in 0.01 M N-ethylmaleimide, the 11S globulin disappeared but no precipitation occurred; instead, a soluble aggregate of 58-67S and a 3-4S fraction formed. It appeared that heating disrupted the quaternary structure; the conversion of soluble aggregates into insoluble ones was catalyzed by sulfhydryl groups but blocked by N-ethylmaleimide, suggesting that hydrophobic interactions are promoted by cleaving the disulfide bonds in the molecule.

Gelation is a denaturation phenomenon of importance in food systems. When soy protein isolates at concentrations above 7% are heated, viscosity increases and gels are formed (79). Gels form within 10 to 30 minutes in the temperature range of 70 to 100°C. Agents capable of reducing the disulfide bond help solubilize the isolates, decrease viscosity of both unheated and heated dispersions and inhibit gelation. Gelation does not occur at low protein concentrations. Aoki and Sakurai (91) reported that soy proteins could be heated in dilute solution, concentrated and reheated to form gels. They prepared isolates by heating meal extracts to 90°C with steam and precipitating the protein with HCl or CaCl₂. Gels formed when the precipitated protein was converted to the proteinate form, diluted to 20% total solids and heated. Sodium bisulfite and 2-mercaptoethanol
inhibited gelation, confirming the results of Circle et al (79) concern- 
cerning the effects of disulfide-cleaving agents on gelation. Disul- 
fide bonds are apparently involved in gelation; sulfhydryl-disulfide 
interchange during heating may result in intermolecular crosslinks 
that stabilize the gel network or intramolecular disulfide linkages 
may help maintain conformation of individual molecules necessary for 
other interactions favoring gelation (73).

d. Solubility of soybean proteins

The behavior of biological macromolecules such as 
proteins in solution is governed by three factors: 1. the size 
and shape of the molecule, 2. the interactions between solvent-solute 
and solute-solute, 3. the charge on the molecule.

Soy proteins appear to have compact structures as 
opposed to the typical random-coil conformations of the milk caseins 
(92). In the case of soy proteins, strong side chain interactions 
cause the molecule to coil up into a hydrodynamic sphere with the 
hydrophilic groups exposed at the surface. Water soluble or salt 
soluble proteins have roughly equal amounts of amino acid residues as 
polar or ionic and non-polar (93).

pH is an important factor in protein solubility be- 
cause the addition of H⁺ or OH⁻ ions can alter the inter- and intra-
attractive forces between molecules, leading to a more stable solu- 
tion (94). Solubility of soybean proteins is also sensitive to salts 
and thus is dependent upon the ionic strength (9).
1. Solvents

A large variety of aqueous solvents have been used to solubilize proteins from defatted soybean meal (95). Water, dilute alkali (pH 7-9) and aqueous solutions of sodium chloride (0.5-2M) are all efficient solvents for different classes of proteins (96, 97). For example, globulins are insoluble at the isoelectric point but are quite soluble if salts are added (96). Because these solvents are relatively inert, the proteins remain undenatured.

2. pH

Distilled water extracts of defatted meal exhibit a pH of about 6.5. If the pH is raised by added alkali, the protein extraction is increased by 5-10%; lowering pH drastically reduces the amount of protein extracted (96). Protein solubility is minimized between pH 4 and 5, the isoelectric region. Extractions can also be made at pH 2-3 but irreversible changes in the 11S protein occur (98).

Extremes of pH, either high or low seem to result in a dissociation of the 7S and 11S globulin into subunits, possibly by electrostatic repulsions between the high positive or negative charges on the proteins; this causes expansion or unfolding of the protein. The subunits are often irreversibly altered by conditions necessary for complete dissociation.

As the pH was lowered from 3.8 to 2.0 at ionic strength 0.06, soybean globulins prepared by acid precipitation no longer presented the usual mixture of 2S, 7S, 11S and 15S fractions, but occurred as 2-3S and 7S fractions. The 11S fraction was present at pH 3.0 and 0.15 ionic strength but at higher ionic strengths, the
fraction was not stable and precipitated from solution. This behavior in acid solution suggested a complex series of association-dissociation reactions influenced by pH and ionic strength. Low pH treatment irreversibly modified 11S protein as measured by ultracentrifuge (98) and by disc electrophoresis (89). Reactions of 11S protein are diagrammed in Figure 1A.

The 7S fraction showed two peaks, 1.92S and 5.47S when pH was lowered to 2.0 by HCl; when the acid solution was dialyzed to pH 7.6, the protein showed a $7S \rightarrow 9S$ monomer-dimer reaction typical of the native protein (68, 69). The reactions are diagrammed in Figure 1B.

In alkaline solutions of pH 12, soybean globulins have been observed to increase in viscosity and gel if the total solids are above 14.5% (74). Conversion of the globulins to the 3S fraction was nearly complete in 15 minutes; this conformational change was irreversible. Disc electrophoresis has shown that alkali-induced changes in the 11S molecule are irreversible (89). Fukushima (75) also demonstrated, by optical rotatory dispersion measurements, that alkali disrupted the internal structure of the 11S molecule. The 7S globulin also undergoes irreversible conformational changes when treated with alkali at pH 12 (68, 69); it is converted to a very slow sedimenting form and adjusting to pH 7.6 ionic strength 0.5 buffer did not restore the 7S form.

3. Neutral salts

The solubility of soy proteins in neutral salt solutions is atypical (97). Such solvents solubilize less soy
A. Reactions of 11S protein. Smith and Circle (9)

B. Reactions of 7S globulin. Koshiyama (68, 69)

Figure 1
protein than water and the solubility curve shows a sharp drop at a
critical concentration that varies with the salt used. Ultracentri-
fuge analysis comparing proteins extracted by different concentrations
of NaCl and CaCl₂ showed that the lowered solubility resulted mainly
from reduced solubility of the 11S and 15S components (99). Reduction
in solubility was more pronounced with CaCl₂.

These effects probably occur because certain
neutral salts are potent general macromolecular denaturants; they can
markedly change secondary-tertiary structure. Ions can be ranked in
order of relative effectiveness in stabilizing or destabilizing the
native protein. For example, in studies with the globular protein
ribonuclease, ions such as SO₄²⁻ stabilize the native conformation,
K⁺, Na⁺, and Cl⁻ have little effect and Li⁺, Ca²⁺, Ba²⁺ and SCN⁻ de-
stabilize it. Since the ions are arranged essentially in the
Hofmeister series that applies to many ionic phenomena, the effects
on macromolecular conformation are probably brought about by general
effects of ions on the structure of the solvent which then modifies
solvent-macromolecular interactions involved in stabilization of the
native structure (100).

4. Solubility related to functionality

Soy protein solubility is related to functional
applications in the food industry.

Metal cations, particularly calcium and magnesium,
combined with heat, have been used for centuries in the Orient to
manufacture a precipitated protein food called tofu. Wolf and Briggs
(101) showed the protein precipitated by calcium ion to be mainly 11S
component.

Several methods are used in industry to measure protein solubility, nitrogen solubility index (NSI) and protein dispersibility index (PDI) being the most common. NSI (102) is known as the slow stir method and requires a two hour dispersion period; PDI is a modification of the NSI method in that only 10 minutes dispersion time is required. NSI value is expressed as the percentage of the total Kjeldahl nitrogen found in the supernatant portion, whereas PDI is expressed as the percentage of total protein extracted with water. Lawhon and Carter (103) have pointed out that the determination of NSI at only one pH is not enough to relate to the overall functionality of the protein in question. Shen (104) stated that the loss of protein solubility cannot be used as the only criterion of denaturation. Chen (105) demonstrated that knowledge of the NSI of soy protein isolates was insufficient to use as an indicator for the functional properties of viscosity, gel strength, water sorption, color and flavor. However, Chen did demonstrate a three-way correlation among the protein extraction conditions, the functionality and the indicators that was believed to be due to processing parameters affecting functionality and the indicators in a similar way. This means that users can specify the functional requirements and manufacturers can produce the proper isolate because the exact processing conditions are known.

D. Soybean Processing

Commercial dry protein fractions of soybeans include full fat and defatted soy flours, refatted soy flours, soy protein concentrates,
soy protein isolates and full fat and defatted soybean milks.

In general, soybean processing consists of a series of steps including seed cracking, separation of seed parts, flaking of the predominantly cotyledonous part, hexane extraction of the lipids in the defatting step, desolventizing of the lipid and defatted portions followed by further fractionation, if desired.

For lipid extraction, n-hexane free of aromatics is commonly used and must be specified for food grade operations (106). A process flow chart is shown in Figure 2.

Soybeans are normally cracked into several pieces before de-hulling, and tempered so the moisture content is 10-11% and the temperature is 160°F (71.1°C) to ensure proper plasticity so acceptable flakes can be formed (107). Temperature and moisture conditions must be carefully controlled to avoid overheating.

Soybean flours are defined as having particles of 100 mesh or less, whereas grits have particles greater than 100 mesh (60). Most soy flours, however, are ground to 200 mesh; some specialty flours are ground until most of the flour passes through a 300 mesh screen. The terms flour and grits as applied to soy refer only to particle size.

Full fat flour is made from the cotyledon without lipid extraction, but, in practice, represents a minor fraction of the commercial edible soybean protein products. To prepare full fat flour, the hulls which are mainly carbohydrate are removed and the dehulled beans ground to yield full fat flour (60). Compositions are shown in Table 5 (108). Soy flour proteins do not have the visco-elastic
Soybeans
  Crack
  Dehull
  Flake

Hypocotyl ("germ")
  Grind
  Classify
  Full Fat Flour

Cotyledon (full fat flakes)
  Desolventize
  Soy Oil
  Degum (Add H₂O)
  Lecithin (emulsion)
  Dry
  Refine

Hulls ("bran")
  Hexane
  Defat
  Defatted Soy Flakes
  Grind
  Classify
  Defatted Soy Flour
  Soy Grits

Smith and Circle (9)

Process flow chart for soybean products.

Figure 2
Table 5. Proximate Analyses of Commercial Soybean Flours and Grits

<table>
<thead>
<tr>
<th></th>
<th>Full Fat</th>
<th>Defatted</th>
<th>Low-Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.0</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Protein N X 6.25</td>
<td>41.5</td>
<td>53.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Fat</td>
<td>21.0</td>
<td>0.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.1</td>
<td>2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Ash</td>
<td>5.2</td>
<td>6.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Meyer (108)
properties characteristic of wheat flours (109). Defatted soy flours and grits are prepared from and retain the chemical composition of defatted flakes.

Proteins, carbohydrates and ash are the major constituents of defatted flour. About one-half of the flour carbohydrates are the oligosaccharides sucrose, stachyose and raffinose while the other half is made up of polysaccharides that are insoluble in water or alcohol (73). Flours and grits are the least refined of soybean proteins; consequently, they have the lowest protein content.

The treatment the flakes receive after they leave the lipid extractor determines many of the properties of the final products and their ultimate use. Flakes leaving the extractor contain about 30% hexane. Its removal is the most critical stage in bean processing because in order to retain protein solubility, control of processing variables with respect to temperature, pressure, moisture and residence time is essential (110).

Soy proteins are sensitive to moist heat and are rapidly insolubilized during steaming (82). The two tests commonly used to measure extent of heat treatment given to flour and grits are the nitrogen solubility index (NSI) and protein dispersibility index (PDI). Although both tests measure the same property, results do not agree because the water extracts are prepared under different conditions (102).

Edible grits and flours are prepared with a range of NSI and PDI values that govern their ultimate use as food ingredients. The range of NSI values for varying heat treatments is shown in Table 6.
Table 6. Variation of NSI with Heat Treatment

<table>
<thead>
<tr>
<th>Extent of heating</th>
<th>NSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>85-90</td>
</tr>
<tr>
<td>Light</td>
<td>40-60</td>
</tr>
<tr>
<td>Moderate</td>
<td>20-40</td>
</tr>
<tr>
<td>Fully toasted</td>
<td>10-20</td>
</tr>
</tbody>
</table>

Smith and Circle (9)
Processes used to desolventize and toast the flakes are listed in Table 7. Flakes desolventized by the Schneckens process have a maximum PDI of only 65-70; normal values are 40-50. Flash desolventization produces flakes of high protein solubility. The vapor desolventizer-deodorizer provides for a very flexible operation and permits production of flakes with PDI values ranging from 10 to 90. Toasted soybean flours of NSI 10-35 are recommended for beverage use because they have optimal nutritive value when compared to dried skim milk (109). After moist heat treatment to the desired NSI, the flakes are dried and ground in a hammermill.

E. Soybean Beverages

A variety of beverages containing soybean proteins have been developed in recent years. Some are designed as infant formulas, some for vegetarian diets and some specifically for developing countries; problems of flavor, acceptability, cost, texture and product stability have been encountered.

1. Traditional soy milk

Soymilk has been one of the staple foods used for thousands of years in the Orient. It is a water extract of soybeans, so-named because of its milky appearance.

Traditional production consists of soaking the whole beans in water overnight, wet grinding the beans, slurrying the ground beans in water, filtering the slurry through a coarse cloth, and then heating the extract to boiling followed by cooling (111).

Soymilk is used to produce a number of other foods, of which tofu is by far the most common. Tofu is manufactured from
### Table 7. Processes Used to Desolventize Soybean Flakes for Edible Products

<table>
<thead>
<tr>
<th>Process</th>
<th>Method of hexane vaporization</th>
<th>PDI range of flakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schneckens</td>
<td>Steam jacketed conveyer with steam sparge</td>
<td>40-50</td>
</tr>
<tr>
<td>Flash desolventizer-deodorizer</td>
<td>Super-heated hexane followed by inert purge gas</td>
<td>70-90</td>
</tr>
<tr>
<td>Vapor desolventizer-deodorizer</td>
<td>Super-heated hexane followed by steam sparge under vacuum or pressure</td>
<td>10-90</td>
</tr>
</tbody>
</table>
soymilk by precipitation with calcium, usually calcium sulfate. After precipitation, the whey is drained off and the tofu cake is washed in water before marketing (9).

The skin that forms on the surface of soymilk heated near boiling is called yuba. Yuba is a film composed primarily of protein and lipid; surface dehydration toughens the film so it can be removed intact and dried (112).

As early as 1925, a medical missionary in China produced and used in nursing homes and hospitals a milk-like product made from soybeans for feeding babies, children and nurses (113). The first commercial development was in Shanghai in 1935. Upon his return to the United States, the missionary introduced into this country soymilk fortified with vitamins and minerals (114). Although the product was intended for general use, it found widest acceptance in feeding infants.

The acceptence of soy beverages produced by the traditional process has been limited in non-Oriental countries because of their characteristic flavor and odor (115). Soybeans ground in the presence of water possess a characteristic flavor that has been described as beany or painty and is generally undesirable. Wilkins et al (116) concluded that the undesirable flavor was the result of lipoxygenase enzyme activity.

a. Lipoxygenase off-flavors

Lipoxygenase acts specifically on fatty acids that contain a cis-cis-penta-1,4-diene system, resulting in the production of cis-trans diene hydroperoxides that have been isolated and
identified (117). In an extensive investigation, Mattick and Hand (118) isolated 80 volatile compounds claimed to be the results of lipoxygenase activity; 40 compounds were identified. The majority of the compounds were aldehydes, ketones and alcohols, many of which had been reported by Fujimaki et al (119). Ethyl vinyl ketone was identified as a primary component of soybean off-flavor.

Lipoxygenase is active only when soybeans are disrupted in the presence of water (120). However, the enzyme system is heat labile; Wilkins et al (116) utilized a hot grinding method for preparation of a soy beverage free of lipoxygenase induced off-flavors. Nelson et al (120) inactivated lipoxygenase by soaking the whole soybean in water until a 100% weight increase was achieved and then boiling the whole bean.

Although heat can be used to inactivate lipoxygenase and eliminate formation of beany flavor, heat can also promote off-flavor development. Some Japanese investigators showed that major flavor components are in a protein bound state (121). These compounds were more readily removed after enzyme-proteolysis. Von Sydow et al (122) demonstrated the heating effect in model systems with soy proteins, SCP and FPC. These proteins could impart undesirable flavors on heating. The binding of flavor compounds to proteins has been reviewed (123). Extraction of hexane-defatted products with polar solvents will also reduce their off-flavor by removing residual phospholipids and numerous minor constituents (124, 125).
2. Methods of manufacture of soybean beverages

a. Cold water extraction

The traditional Asian preparation of soymilk is a cold water extraction procedure of water soaked and ground soybeans. Since lipoxygenase has not been inactivated, lipid oxidation occurs with subsequent development of the characteristic painty beany flavor.

Extraction by filtration removes the insoluble material and the resulting milk is a highly stable oil emulsion with a smooth texture (116). Subsequent boiling inactivates trypsin inhibitor, thereby improving nutritional value.

Hackler et al (126) reported that time and temperature of heat treatment of soymilk from 1 to 6 hr. at 93°C had no adverse effects on PER or available lysine. However, brief cooking at 121°C decreased available lysine. Spray drying with an inlet temperature of 277°C or above caused a drop in nutritional value and available lysine.

b. Hot water extraction

Bourne et al (127) evaluated modifications of the traditional method. Heating the beans before grinding inactivated lipoxygenase thus preventing painty flavor development but prefiltration heating resulted in very low yields of soymilk. If the soaked beans were ground in boiling water, while maintaining a temperature of at least 80°C in the grinder at all times, filtered, the filtrate boiled 30-60 minutes to inactivate trypsin inhibitor, sweetened and flavored, a product free of lipoxygenase induced off-flavors was obtained.
Wilkins et al (116) found that they could produce a nearly bland milk by using high temperatures (above 80°C) and a rapid hydration procedure on dehulled soybeans. Lower temperatures could be used for grinding if sufficient antioxidant, nordihydroguaiaretic acid, was added to the grinding water. Lao (117) found that other antioxidants, including BHA, BHT, and citric acid did not prevent lipid oxidation during grinding at 65-75°C.

Maximum yield of soymilk and solids was by extraction in the range of 50 to 75°C (116, 128, 129, 130). At grinding temperatures above 75°C a significant reduction in solids yield was observed, due to hindered filtration by protein gelation or swelling of the insoluble carbohydrate fraction. McMillan (131) presented evidence that retention of extractable solids by the filter cake during filtration was caused by a combination of absorption and adsorption on the fibrous material.

c. Acid extraction

It has been reported that if soaked dehulled soybeans are extracted at 25°C at pH 3.0 or below, a bland soymilk may be obtained (130) in which lipoxygenase inactivation is complete and irreversible. To maximize yield, it may be necessary to extract at pH 2.0.

Kon et al (132) observed that a bland soymilk was obtained by extraction of soybean with water at pH 3.85 or below. The manufacturing process consisted of grinding dry soybeans with acidified water, filtering the slurry, cooking the filtrate for 1 hour and adjusting pH of the beverage to 6.5.
Lao (117) found that extraction at pH 2.0 yielded a soymilk free of painty flavor. However, the beany flavor present seemed to be inherent to soybeans and not due to lipoxygenase activity.

d. Alkaline extraction

Al-Kushtaini (130) prepared a bland soymilk by extraction at pH 9.5 and 25°C. However, lipoxygenase activity was still present, suggesting that more than one enzyme was responsible for oxidized off-flavor development.

Badenhop and Wilkens (133) showed that 1-octen-3-ol is formed in soymilk when high temperature grinding is preceded by an initial water soak, but the amount formed varied with pH and other factors, with the amount formed decreased in alkaline solution. In a more detailed study, Badenhop and Hackler (134) evaluated effects of soaking in NaOH prior to boiling water extraction. Pretreatment in 0.05N NaOH produced a milk of pH 7.37, that was judged to have a significantly better flavor. A higher niacin content was also found. Although there was a slight loss in cystine, this was not considered to be serious in view of the improved flavor.

Bourne et al (135) studied the effects of adding various salts and alkalis on pH and flavor of soymilk. They showed that NaOH at pH 7.0 and 7.5 had higher acceptability but samples at pH 8.0 were judged soapy. Soymilks adjusted to pH 7.0 and 7.5 with Na₂CO₃ and NaHCO₃ were inferior and the investigators suggested that flavor differences were related to the sodium ion concentration rather than pH differences. They then prepared soymilks at equimolar
sodium ion concentrations, using a variety of sodium salts; results showed that differences in acceptability between sodium ion concentrations were highly significant whereas differences due to compounds were not. NaCl was not evaluated because the salty flavor masked effects of the sodium ion.

Kuntz (136) extensively studied the processing factors affecting the organoleptic quality of the product, known as "Illinois soy beverage." He reported that optimal beverage quality was obtained by blanching cotyledons in alkaline medium (0.25% NaHCO₃), maintaining pH at 7.5 throughout processing, homogenization of milled soy slurry and beverage at 180°F (82.2°C) and 3500 psi (246.1 kg/cm²), adjustment of TS to 6% and addition of 0.1% added NaCl.

e. Protein-lipid concentration

Mustakas (137) prepared a soy beverage by means of a protein-lipid concentrate (LPC). Both insoluble and soluble carbohydrate fractions were removed in what amounted to production of a full fat protein isolate. LPC was isolated from full fat flour by an acid wash at pH 3.5; the acid curd was heated to inactivate lipoxygenase, centrifuged to remove water soluble constituents, resuspended in alkaline medium (pH 9.0), heated by steam injection to 205°F, cooled and adjusted to pH 7.2. The mixture was then fed to a colloid mill, homogenized at 8000 psi (562.6 kg/cm²) and centrifuged to remove insoluble material.

Mustakas (137) reported the presence of bound lipids, that is, that portion of total lipid not extracted by pentane hexane, in a freeze-dried LPC sample. The amount ranged from 13-24% of the
total lipid. It was postulated that the bound lipids were in the lipo-protein or glyco-lipid form.

f. Non-extractive methods

Non-extraction procedures for soybean beverage preparation incorporate essentially the whole soybean or cotyledon into the beverage. The whole bean, as is, or a powder or flour may be used. There is no separation of insoluble material by filtration although some processes use centrifugation to remove insoluble material.

Hand et al (115) and Van Buren et al (138) have made extensive pilot plant studies of suitable equipment and processes for manufacturing soymilk. They reported that the classical water extraction procedure yielded the best product with regard to flavor and consistency. They also found essentially the same PER regardless of whether the milks were spray dried, vacuum roller dried, atmosphere roller dried or freeze dried.

Hand et al (115) described two methods for soymilk preparation. The first used whole beans, steamed 45 min. at atmospheric pressure and then dried 10 min. by forced air at 220°F (104.4°C). The beans were dehulled, the hulls removed, and the cotyledons were ground and the flour slurried with water to 16% TS. The slurry was homogenized at 2000 psi (140.6 kg/cm²) and spray dried to make a reconstitutable beverage powder.

In the second method, soybeans were steamed 2-5 min. at 212°F (100°C), heated 10 min. at 220°F (104.4°C) in forced air, dehulled, hulls removed and the resulting cotyledons soaked overnight. The drained soaked cotyledons were steamed 45 min. at 212°F (100°C),
ground with water at 16% TS, homogenized and spray dried as before. Nelson et al (139) evaluated these products and reported that they had poor throat and mouth feel quality.

Miles (140) prepared a soybean beverage from flaked cotyledons. The flakes were slurried in water and a phosphate stabilizer or a sequestering agent like EDTA added to prevent protein coagulation during heat processing. The slurry was pressure cooked at 220-250°F (104.4-121.1°C) for up to 10 min. and homogenized at 5000 to 8000 psi (351.6 to 562.6 kg/cm²). The slurry was "clarified" with a centrifugal separator, followed by formulation and homogenization at 2750 psi (193.4 kg/cm²).

Lo (141) prepared a beverage from a full fat soy flour. The flour was prepared from soybean cotyledons that were flaked, extrusion cooked, toasted and ground to 270-300 mesh. The beverage was prepared by adding water containing a stabilizer, carrageenan. The resulting slurry was heated to 180-190°F (82.2-87.8°C) and held for 30 minutes prior to homogenization at 8000 psi (562.6 kg/cm²). Bottling and sterilization followed.

Lo also utilized a continuous centrifuge to remove some of the insoluble carbohydrate. Clarification was followed by homogenization at 2500 psi (175.8 kg/cm²), bottling and sterilization.

Lo's process has been the basis for the first successful commercial effort for promotion of soymilk (142). The product is marketed in Hong Kong under the trade name "Vitasoy." This product contains about 2.5% protein and competes quite successfully with soft drinks.
Mustakas et al (143, 144) developed a soy beverage process utilizing either full fat or defatted enzyme-inactive soy flour. The process is one of producing a spray dried beverage powder easily reconstituted by adding water. The powder was made by slurry-ing soybean flour with water to produce a 10 to 20% solids slurry. The slurry was wet-milled by passing through a colloid mill set at 0.001 inch, and homogenized at 3500 psi (246.1 kg/cm²) followed by spray drying. Use of an emulsifying agent such as polyoxyethylene sorbitan monostearate (Tween 60) was also specified to improve suspension characteristics.

Berra and Pontecorvo-Valhuerdi (145) reported use of a beverage in Mexico that was developed by Castello of Secado Artificial S.A. The beans were soaked and boiled, milled with additional water and the resulting slurry was emulsified to make the beverage.

Nelson et al (139, 146) developed a process to prepare a soy beverage from whole soybeans, with or without hulls. (Illinois process).

Whole soybeans were tenderized by blanching in boiling water usually with prior soaking, while cotyledons were tenderized only by blanching. Usually NaHCO₃ was present in the soak and blanch solutions. After blanching, the soy solids were milled with additional water in a hammer mill, followed by heating the slurry to 200°F (93.3°C) and homogenizing at 3500 psi (246.1 kg/cm²). The resulting beverage base was diluted with water to the desired TS, sweeteners and flavoring were added and beverage pH was adjusted to approximately
7.2. The formulated beverage was heated to 180°F (82.2°C), rehomogenized at 3500 psi (246.1 kg/cm²) and bottled.

Blanching the soybeans before grinding inactivated lipoxygenase so the beverage was free from lipoxygenase induced off-flavors (120). Soluble carbohydrates were leached from the intact beans or cotyledons during soaking and blanching (147). The Illinois beverage had good mouth feel and suspension stability (146).

Kuntz (136) made an extensive investigation of the mechanisms affecting organoleptic quality of the Illinois soy beverage. Chalkiness in the finished beverage was related to the presence of suspended insoluble material; removal of the particulate material reduced chalkiness intensity to the imperceptible. Greatest correlation with chalkiness was found with that fraction of insoluble material retained by a 150 mesh screen. Astringency and cereal flavor were also significantly reduced by removal of the beverage insolubles; the effect of desludging on bitterness was not significant. Analysis of the sludge showed the bulk of it to be particulate fiber fragments.

Hsieh et al (148) developed a rapid and efficient process for soy milk in which whole soybeans were ground to yield particles 420 to 590 micrometers in diameter, dispersed in hot water (1 part to 11 to 13 parts of water) at 85 to 95°C and mixed 30 minutes. Next, the mixture was homogenized at 3000-4000 psi (211.0-281.3 kg/cm²); the resultant slurry was adjusted to pH 5 for enzyme treatment with pectinase, cellulase and hemicellulase to digest the hull. The enzyme treated slurry was adjusted to pH 7 and rehomogenized twice at 6000-7000 psi (421.9-492.3 kg/cm²) and sterilized. The
product was bland in flavor and had good mouthfeel, the improvement being related to the fact that the average particle size was reduced to below 2 micrometers by this treatment.

3. Beverages containing cheese whey and soybeans

Holsinger et al (149) have previously reviewed the use of cheese whey as a base for the manufacture of a variety of beverages. Since soybean protein is low in the sulfur-containing amino acids and since whey proteins are particularly rich in lysine and contain significant amounts of methionine and cystine their combination offer an excellent potential for development of products of high nutrition/cost ratio. Soy-whey beverages with a protein content equal to or greater than that of milk would be desirable additions to the available food supply.

a. Soy-whey milks

Tsugo (150) described a process whereby sweet whey or neutralized acid whey and soybean milk were condensed to produce a product resembling evaporated milk. A soy-whey milk prepared from soybeans and cottage cheese whey was developed at the University of Illinois (151). The beany flavor was destroyed by boiling the whole bean before grinding for incorporation into the whey. The product had a flavor resembling egg nog.

Lowenstein and Paulraj (152) demonstrated that a powder made by coprecipitating and drying defatted soybean flour and whey protein to a final blend of 3 parts soy to 1 part whey protein was clearly superior to soy protein alone in promoting growth of rats.
Sasaki and Tsugo (153) described the manufacture of synthetic milk powder from whey and soybeans by extraction of the beans with hot whey. This research led Guy et al (154) to develop a process for spray drying a mixture of full fat soy flour and fluid sweet whey to yield a free flowing powder of good nutritive value suitable for beverage use. The powder contained 67% sweet whey solids and 33% full fat soybean flour. Another powder containing 55% sweet whey solids, 28% soy flour and 17% corn oil was also manufactured by this process.

To manufacture the powder, liquid sweet whey was combined with full fat soy flour and then pasteurized continuously at 77°C for 20 sec, homogenized in 2 stages using pressures of 387 and 38.7 kg/cm² and condensed to 40-50% TS in vacuum and spray dried in conventional fashion or by using air injection (155). The formulation of the product was flexible and permitted addition of sweetener and flavorings so that a beverage containing 2.7% protein was obtained. Citrus and cherry-vanilla flavors were highly acceptable to a trained taste panel (156).

Homogenization reduced the amount of settling of the soybean solids and yielded a suspension more like milk. Concentration under vacuum and spray drying reduced the beany flavor of the soy bean flour.

A spray dried beverage powder containing skim milk whey and whole soybeans was developed in Canada (157).
b. Whey-soy drink mix

The preparation of whey soy drink mix (WSDM) for use in the U.S. Food-for-Peace program is the basis for the present study. The product (158) manufactured under specifications (159) and intended as a nonfat dry milk replacer, is formulated from sweet cheese whey, soy flour (full-fat, FDI 35-45 or fully toasted, defatted, NSI 10-30), soybean oil and 42DE corn syrup solids. After formulation, the mixture is homogenized double stage at a pressure greater than 141 kg/cm² for the first stage and 35.2 kg/cm² for the second stage, pasteurized at 79.4°C for 25 sec, condensed under vacuum and spray dried using the techniques developed by Guy et al (154). 1% of a vitamin mineral premix may be dry blended into the powder if desired.

The addition of whey to the formulation significantly improved the nutritional quality of the soybean flour. WSDM had a PER of 2.1 compared to reference casein at 2.5. The net protein utilization value was 75% compared to a value of 34% for casein (158). The PER of soybean protein averages 1.8 or below (160). The amino acid pattern of WSDM is shown in Table 8.

Although the product was rated acceptable during extensive testing in developing countries (161) some unsolved problems remained. A contract was let to a commercial firm to study means of overcoming two problems: objectionable soy flavor and grittiness (162, 163). The intensity of soy flavor could be reduced by heating, but the soy flavor was replaced by a cooked flavor unless heating was carefully controlled. It was concluded that the best solution was to flavor the product with artificial milk flavors. Use of a defatted
Table 8. Amino Acid Pattern of WSDM

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>g amino acid/100 g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>7.35</td>
</tr>
<tr>
<td>His</td>
<td>2.22</td>
</tr>
<tr>
<td>Arg</td>
<td>6.49</td>
</tr>
<tr>
<td>Asp</td>
<td>12.37</td>
</tr>
<tr>
<td>Thr</td>
<td>5.10</td>
</tr>
<tr>
<td>Ser</td>
<td>5.57</td>
</tr>
<tr>
<td>Glu</td>
<td>19.74</td>
</tr>
<tr>
<td>Pro</td>
<td>5.21</td>
</tr>
<tr>
<td>Gly</td>
<td>4.07</td>
</tr>
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<td>Ala</td>
<td>4.64</td>
</tr>
<tr>
<td>Cys</td>
<td>.72</td>
</tr>
<tr>
<td>Val</td>
<td>5.66</td>
</tr>
<tr>
<td>Met</td>
<td>3.69</td>
</tr>
<tr>
<td>Ileu</td>
<td>6.86</td>
</tr>
<tr>
<td>Leu</td>
<td>9.48</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.89</td>
</tr>
<tr>
<td>Phe</td>
<td>5.03</td>
</tr>
</tbody>
</table>
soy flour in the formulation improved the suspension stability of the reconstituted beverage, and reduced grittiness, apparently due to greater ease of dry grinding the defatted flour to a finer particle size. The investigators also claimed that using a defatted soy flour of NSI 45 improved suspension stability with no flavor impairment. They also recommended the use of Tween 60 to improve dispersibility during reconstitution of the finished powder.

Research has continued at the USDA Dairy Laboratory on the effects of alteration in manufacturing procedures on the physical properties, flavor and storage of WSDM. Cottage cheese whey was evaluated as an ingredient in WSDM (164). Samples prepared with neutralized acid whey or with a 1:1 mixture of acid to sweet whey showed decreased flavor score both initially and during storage. These samples contained over 1% lactate reported as lactic acid; decreased flavor score of commercially produced WSDM was correlated with the lactic acid content of the powder. Findings showed that if WSDM was manufactured with only small amounts of acid whey, storage stability as measured by flavor acceptability could be impaired.

Substitution of either 26 to 29 DE corn syrup solids or 9 to 12 DE dextrin for 42 DE corn syrup solids was also evaluated for effects on nutritional quality and flavor of WSDM (165). Results showed that although substitution had no deleterious effects on protein quality and digestibility during 1 year of storage at 37°C, only the 26-29 DE corn syrup solids provided the required flavor qualities.

An oil fraction from edible beef tallow (BTOF) was evaluated as a substituent for the soybean oil in the WSDM formulation
The BTOF had characteristics similar to soybean oil with greatly increased stability to oxidation; its AOM (active oxygen method) stability was 238 hours compared to 23 hours for the soybean oil used. Particular attention was paid to the physical properties related to rehydration of WSDM containing BTOF. Sinkabilities and bulk densities of the powders containing BTOF were greater than those containing soybean oil; since densities of the oils used in product formulation were virtually identical, results suggested that the structure of the dried particles was different. The dispersibilities were the same as the controls, however.

Other processing parameters have also been evaluated for their effects on the physical properties of WSDM (167). When medium heat sweet whey powder was reconstituted to 16% TS and processed using either the full fat or defatted flour formulation, powders containing 3.25% moisture or less could not be obtained until the TS of the concentrate fed to the dryer was reduced from 40 to 32%. Dispersibility and sinkability of the powder prepared from reconstituted sweet whey solids were both reduced whereas solubility index and bulk density were both increased. No flavor problems were caused by ingredient substitution.

Instantizing by foam spray drying by nitrogen injection, in which gas is injected into the high pressure feed line leading to the spray dryer (155), had no effect on dispersibility but greatly reduced both sinkability and bulk density. Foam spray drying reduced sinkability because of entrapment of gas in the powder particles. Foam spray drying was necessary to reduce product buildup
on the hot surfaces of the spray dryer if sweetness was increased by pretreating the whey used in the formulation by β-galactosidase (lactase) enzyme to hydrolyze 70% of the lactose present to the constituent monosaccharides glucose and galactose. In this case, foam spray drying did increase the dispersibility of the finished powder. Flavor acceptability was improved by enhanced sweetness of the reconstituted beverage.

4. Effects of homogenization on soybean beverages

Homogenization is a standard industrial process practiced as a means of stabilizing a fat emulsion against gravity separation. In the case of milk, its normal characteristics are markedly affected by homogenization. Although the most obvious changes occur in the lipid phase, changes also occur in the plasma proteins that affect the overall characteristics. Homogenized milk is blander in taste, whiter in color, less heat stable, more viscous, and has greater foaming capacity and lower curd tension than unhomogenized milk (168).

Most homogenization is carried out by forcing milk through small passages under pressure at velocities of about 600 to 800 ft/sec (182.9 to 243.8 meters/sec) (169, 170). Fat globules in unhomogenized milk vary considerably in size and show a tendency to cluster together, whereas fat globules of properly homogenized milk are small, more uniform in size and the state of their dispersion remains fixed. For raw milk, Cornell and Pallansch (171), using a Coulter counter technique, showed that over 95% by weight of the fat consisted of globules with diameters between 1 and 10 micrometers. After homogenization at 176 or 282 kg/cm², only a few per cent by weight were larger
than 2 micrometers and a significant part occurred as globules with
diameters less than 0.3 micrometers.

Homogenization appears to be an essential step in the
manufacture of many soybean beverages. The literature is filled with
references to effects of heat treatments and pH during processing on
the functionality of soy proteins but little information is available
about the effects of homogenization on properties of the soybean pro-
teins and products prepared containing them.

Mustakas et al (143) claimed that coarse gritty particles
in the extruded full fat flour (172) used in product formulation
caused poor mouth feel when the flour was suspended in water. The
combined steps of colloid milling and homogenizing reduced the par-
ticle size so that mouth feel was much improved. Spray drying re-
formed some granular particles but they were minimal if spray drying
was carried out at low temperatures.

SEM photographs of these products showed the original
flour to be aggregated in clusters about 75 micrometers in diameter.
After water dispersion, colloid milling, homogenization and spray
drying, size degradation to 20 micrometers or less had occurred.

Mustakas (137) reported that good to excellent mouth feel
in the reconstituted LPC beverage could be attributed to the reduction
of the lipid complex curd to fine particles. He also claimed that
reducing the curd to small particle size (<20 micrometers) contributed
to the excellent suspension properties. If colloid milling and
homogenizing were omitted during product manufacture, SEM showed the
spray dried particles to be more agglomerated; there was more solid
separation in the clarification step and poor mouth feel and sedimentation after reconstitution. High pressure homogenization (8000 psi; 562.6 kg/cm²) reduced particle size and gave beverages with better mouth feel than low pressure homogenization (3500 psi; 246.1 kg/cm²).

Nelson et al (146) investigated the interaction between number of passes through the homogenizer, homogenization pressure and slurry temperature when homogenized on mouth feel and colloidal stability of "Illinois" soybean beverage. A single homogenization at 200°F (93.3°C) gave a good product provided homogenization pressure was at least 6,000 psi (421.9 kg/cm²). Double homogenization at 3,500 psi (246.1 kg/cm²) and 60°F (15.6°C) gave acceptable mouth feel and colloidal stability.

A combination of hot (200°F) (93.3°C) and then cold (60°F) (15.6°C) homogenization or hot-hot homogenization resulted in a beverage of better colloidal stability and mouth feel than a cold-cold homogenization. If a 1,500 psi (105.5 kg/cm²) initial pressure was used, a second pressure of 5,000 psi (351.6 kg/cm²) was needed for a stable product; likewise, with an initial pressure of 2,500 psi (175.8 kg/cm²), a good product was obtained with 4,000 psi (281.3 kg/cm²) second pressure. An important consideration was the additive effect of the two pressures.

Priepeke (173) confirmed a significant linear relationship between the sum of pressures of all homogenizations and suspension stability. The minimum sum of pressures required for suspension stability was 5,000 psi (351.6 kg/cm²) when homogenization was at 180°F (82.2°C). This could be achieved with a single pass. Increased
temperature of homogenization enhanced suspension stability; high
temperature of homogenization could compensate for low homogenization
pressure and vice versa.

Kuntz (136) showed that homogenization conditions had a
significant effect on the flavor and tactual quality of the "Illinois
soy beverage." This author proposed a relationship between chalkiness
and particle size in the beverage.

Protein bodies in water blanched samples imbibed more
water and were more susceptible to disruption during homogenization.
Products homogenized at higher temperatures were less chalky. The
size of proteinaceous particles was much smaller with high tempera­
ture homogenization. Higher homogenization pressures also resulted
in more extensive disruption of proteinaceous material. The author
identified large protein-fiber agglomerates in products from which
lipids had been removed by extraction; these beverages had pronounced
chalky flavor and poor suspension stability. The insoluble carbohydrate fraction was identified as being related to degree of chalki­
ness in the beverage. This fraction of the beverage was more exten­sively hydrated by processing in alkaline conditions and heating to
higher temperature before homogenization. Therefore, this fraction
was more completely disrupted by grinding and homogenization; disrup­tion was enhanced by increasing homogenization pressure. Therefore,
the conditions resulting in smaller particle sizes not only improved
suspension stability, but produced beverages less chalky and
astringent.
Hsieh et al (148) applied extremely high homogenization pressures (6,000-7,000 psi) (421.9-492.3 kg/cm²) to an enzyme treated slurry in order to ensure good mouth feel of the sterilized end product. They reported that in the finished product, 90% of the particles had a diameter of less than 2 micrometers as measured by Coulter Counter. No suspension stability data were reported, however.

When a whole soybean milk base was spray dried, microscopic examination of the dried particles showed a reduction in particle size attributed to homogenization (174). It was claimed that homogenization produced smaller particles which were apparently suspended more easily; the smaller particles may have produced smaller droplets upon atomization. Smaller droplets should dry faster, require less heat and be more soluble (175).

These authors also observed a decrease in protein dispersibility index of the reconstituted powder that had been subjected to homogenization pressures above 6,500 psi (457.1 kg/cm²) during processing. They claimed this decrease was due to adverse effects of high pressure on the soy proteins themselves because homogenization of cows' milk results in decreased heat stability and solubility; the effects for cows' milk also increased with increased homogenization pressure. If the soy milk base was frozen and thawed before spray drying, the homogenization pressure used before freezing had no effect on the protein dispersibility of the powder which was reduced as a result of freezing. When the frozen and thawed base was rehomogenized double stage at 3,500-500 psi (246.1-35.2 kg/cm²) before spray drying, no increase in PDI was observed, showing that rehomogenization
did not lead to resuspension of the insolubilized proteins.

Guy et al (154) demonstrated that homogenizing greatly improved the dispersion of soy flour solids in whey; this resulted in drastic improvement of the rate of particle settling upon reconstitution of the spray dried product. Poorly dispersed solids remaining in the whey-soy flour mixtures after homogenization were shown to be soy components.

These authors also demonstrated that homogenization had little effect on promoting fat-protein interactions leading to low free fat levels when soy flour alone was homogenized and shelf dried. However, whey seemed to contain an interfacially active material which, on simple mixing of whey and soy-flour, followed by homogenizing and shelf drying, protected the soy lipids from solvent extraction. As found with cows' milk (176), the method of drying affected the free fat content significantly. Spray drying yielded lowest levels of free fat.

5. Effects of processing conditions on viscosity of soybean beverages

Rheological properties of proteins (intrinsic viscosity, apparent viscosity) can often reflect the conformation of the proteins. Soybeans are globular proteins; their intrinsic viscosity is generally lower than that found for flexible polymers (e.g., polystyrene) of comparable molecular weight. It is believed that rigid compact proteins will move through the solvent more easily than swollen chains (177); however, the intrinsic viscosity of rigid particles is dominated by particle asymmetry, not size. Shen (178) used the intrinsic
viscosity to demonstrate that the soluble proteins of an acid precipitated curd and a commercial soy protein isolate were essentially in the native globular state.

Data regarding the effects of processing conditions of soybean beverages on their viscosity are somewhat sparse. For example, the viscosity behavior of soy milk during concentration has been investigated by very few workers.

A serious problem arises in the concentration of soy milk because of a sharp increase in viscosity as the total solids approaches 30% (179). Lo et al (180) reported a logarithmic relationship between the apparent viscosity of soy milk and the total solids content. Using a Brookfield viscometer, at 5% TS, the viscosity was directly related to the speed of rotation of the spindle, indicating that soy milk is non-Newtonian. At 20% solids, the apparent viscosity decreased with increasing time of shear, demonstrating the thixotropic nature. Upon concentration to 27% TS the soy milk gelled.

Forewarming of cows' milk for various times and temperatures is effective in controlling viscosity of evaporated milk (181, 182, 183). However, Tan (179) reported that when soy milk was forewarmed before concentration, both time and temperature of forewarming increased viscosity of the concentrated soy milk. Lo et al (180) demonstrated that treatment of the soy milk with sodium sulfite, a disulfide bond disrupter, at levels above 630 ppm before evaporation reduced the apparent viscosity of condensed soy milk at solids content below 20%, above which the viscosity increased logarithmically.
Formation of disulfide bonds may cause viscosity increases and formation of gel-like structures in aqueous dispersions of soy proteins (79). Ferry (184) suggested that gel formation came about by proteins denaturing and unfolding, permitting exposure of reactive sites as well as non-polar groups. During gel formation, cross-links could be formed between active sites along the polypeptide chains. Sodium sulfite treatment reduced viscosity of isolated soy proteins (79, 180). Therefore, thickening during soy milk concentration could be partly due to formation of disulfide bonds.

Apparent viscosity increased with increasing protein isolate concentration and pH during spinning of fibers (174). Ishino and Okamoto (185) examined dependence of molecular interactions in alkali denatured soybean proteins on pH and protein concentration. Their results showed that the proteins underwent a conformational change above pH 11; viscosity increases or gelation were strongly inhibited by treatment with urea or mercaptoethanol, showing that disulfide linkages were involved. Cystinyl residues decomposed near pH 11. Interaction during dialysis to pH 7.2 after treatment above pH 11 resulted from intermolecular bonding (disulfide, hydrophobic, hydrogen) across reactive sites made accessible by unfolding of the polypeptide chains during alkali treatment. Undialyzed proteins treated at pH 7.2 and 10.4 showed high viscosities attributed to protein aggregation and increased hydration rather than conformational changes.

Mustakas et al (143) reported the viscosity of a 10% aqueous dispersion of their high protein beverage base to be slightly higher than cows' milk (6.4 vs. 4.2 cp at 20°C). However, if the solids content was raised to 12.5%, the viscosity doubled (8.1 vs.
Mustakas (137) found a sharp increase in viscosity during the secondary alkaline cook required for complete trypsin inhibitor inactivation during manufacture of the LPC beverage base. Five minutes cooking at 205°F (96.1°C) gave a Brookfield viscosity of 18 compared to 6.5 after 1 minute's cooking. Fortunately, 1 minute of cooking at 205°F (96.1°C) at pH 9 was sufficient for 100% trypsin inhibitor inactivation.

Although Nelson et al (146) examined effects of homogenization on suspension stability and mouth feel of "Illinois soybean beverage," they showed no data relating viscosity to homogenization conditions. They did show that soybean protein concentration or presence of hull appeared to have no effect on suspension stability; both increasing protein concentration and increased levels of fibrous hull increased viscosity. However, they inferred from this that beverage stability was not related to viscosity.

In contrast to Nelson et al (146), however, Priepke (173) reported that increased viscosity of the "Illinois beverage" had a favorable effect on suspension stability. Solids stability was particularly enhanced at protein levels of 3.8% and higher. Priepke also reported that addition of both sucrose and sodium chloride favorably affected total solids suspension stability but had no effect on protein suspension. Density was increased only slightly. Although sucrose increased viscosity, sodium chloride decreased viscosity so viscosity alone did not account for improved TS suspension stability.
A detailed examination of factors affecting viscosity of the "Illinois soybean beverage" was carried out by Forster and Ferrier (186). They showed that rheological properties of the beverage were adequately described by the general power law equation
\[ \tau = b\gamma^n \]
where \( \tau \) = shear stress, \( \gamma \) = shear rate, \( b \) = consistency coefficient and \( n \) = flow behavior index. The beverage displayed pseudoplastic flow behavior and was mildly thixotropic. As total solids increased, apparent viscosity and degree of pseudoplasticity increased. Presence of soybean hulls in the beverage increased apparent viscosity over that found for a beverage prepared from de-hulled cotyledons. As hull concentration increased, the greater was the deviation from Newtonian behavior. The beverage homogenized twice, double stage at 3,500-500 psi (246.1-35.2 kg/cm²), had lower apparent viscosity and greater adherence to Newtonian behavior than the beverage homogenized once at 3,500-500 psi (246.1-35.2 kg/cm²).

The authors believed the apparent viscosity was lower in the double homogenized beverage because the mean particle size was decreased by the second homogenization (136). Smaller particles would more readily orient in the direction of the shearing force, thus lowering viscosity and reducing deviation from Newtonian behavior.

Beverages prepared from cotyledons blanched in sodium bicarbonate had higher apparent viscosities and were more pseudoplastic than beverages prepared from cotyledons blanched in an acid solution as in tap water. Bourne et al (135) reported that greater hydration of soy proteins resulted if the blanch solution contained sodium ions; therefore, addition of sodium bicarbonate may have
resulted in increased swelling of protein molecules and therefore increased protein solubilization and viscosity as reported by Forster and Ferrier (186).

Guy et al (154) demonstrated that when cheese whey solids were added to aqueous dispersions of soy flour, interactions between components of the two ingredients prevented the excessive viscosity increases observed on concentration of aqueous dispersions of soy flour alone. If soy flour suspensions and wheys were separately concentrated and then blended together, the observed specific viscosities of the mixtures were always higher than if the components were first blended together and then condensed. The specific viscosities of soy flour-whey mixtures were relatively unaffected by normal heat treatments; however, if the TS of the system was above 45% TS, then significant viscosity increase could be associated with increased heat treatment.

Homogenization of the slurries decreased specific viscosity resulting in decreased solubility index of the finished powder. As homogenization pressure increased from 2,000 to 5,500 psi (140.6 to 386.8 kg/cm²) both viscosity of the resultant concentrate and solubility index of the dry powder decreased markedly. Therefore, the addition of whey to aqueous dispersions of soy flour not only improved nutritional quality but made the handling of 50% total solids containing mixtures feasible in manufacturing operations.

6. Suspension stability of soybean beverages

Little definitive work in the area of factors affecting suspension stability of soybean beverages, particularly reconstituted
spray dried ones, has been carried out. Some beverages tend to whey off, that is, form a clear serum layer at the top of the beverage, which may or may not be accompanied by flocculation and settling of the solids. Sometimes no clear serum layer is formed but differences can be found in solids composition between the top and bottom of the container. Additionally, oiling off can occur, that is, formation of a lipid or lipid-protein layer at the top of the beverage.

Hand et al. (115) implied suspension stability of their reconstituted spray dried soybean milk. Grittiness of their reconstituted spray dried acid precipitated curd was objectionable.

Miles (140) made no specific statements regarding suspension stability of his beverage. However, some problem must have been encountered because he recommended addition of phosphate stabilizer or EDTA to the slurry before cooking, and centrifugation after high pressure homogenization.

Lo (141) combined high pressure homogenization with addition of carrageenan to produce a stable suspension; he also recommended centrifugation to remove coarse particles. However, centrifugation also removed 30% of the soy flour solids from the beverage.

Mustakas et al. (143) gave no indication of beverage stability. However, they claimed that suspension properties of the spray dried beverage base in water were improved by the addition of 0.8% Tween 60 emulsifier during product formulation and by resuspending in hot water (150-160°F) (65.6-71.1°C).

Mustakas et al. (144) described essentially the same beverage reported in 1971. Again, no description of beverage
stability after reconstitution was given.

The LPC beverage described by Mustakas (137) was claimed to have better suspension properties than the 1971 beverage. Stability was described as "no phase separation" or sediment after 7 days of standing after reconstitution. Both colloid milling and homogenizing were employed during powder manufacture.

Nelson et al (139) compared suspension stability characteristics of the "Illinois beverage" with those of Hand et al (115), Miles (140), Lo (141) and Mustakas et al (144) and reported that all showed unstable suspensions after varying lengths of time.

Nelson et al (146) claimed that the "Illinois beverage" showed excellent suspension stability after 2 months of refrigerated storage. A Coulter Counter study of particle size distribution showed no particles below 2.7 micrometers in diameter and only about 10% larger than 10 micrometers. Since these particle sizes fell above the colloidal range, the investigators concluded that particle size alone was not the controlling factor in suspension stability. They believed that suspension stability of the "Illinois beverage" was due to formation of hydrophilic lipid-protein complexes caused by the combined effect of tenderization of intact cotyledons and homogenization of the resulting slurry. They suggested that during tenderization, the soybean proteins and carbohydrates were hydrated such that the protein became susceptible to protein-lipid complex formation. They also suggested that the resulting complex was comprised of protein surrounding a core of soybean oil such that the hydrophilic phospholipids migrated to the surface of the complex and
thus created a water sheath around the normally hydrophobic protein. Because of the oil core, the complex particle would be less dense than normal protein, thus maintaining suspension stability. Nelson et al (139, 146) also reported the occurrence of bound lipid in the "Illinois beverage." They believed that complexed lipid must be greater than 50% of the soybean oil if an acceptable beverage was to be obtained.

Priepke (173) made a detailed study of the suspension stability of "Illinois soybean beverage." He investigated both the effects of processing steps and conditions and the role of the lipid component on suspension stability. He defined suspension stability as a maintenance of a uniform dispersion of solid and lipid particles in the beverage during quiescent storage. He reported that minimum tenderization required for resultant beverage stability was achieved after soaking whole beans overnight in 0.5% sodium bicarbonate followed by 10 minutes blanching in the same solution; ideal tenderness required 30 minutes blanching. Cotyledon tenderization by direct blanching required 30 minutes in 0.25% sodium bicarbonate. Both increased blanch time and increased concentrations of sodium bicarbonate improved beverage suspension stability. Slurry pH had to be slightly alkaline at the first homogenization for best suspension stability in the beverage.

When the final beverage was formulated, increased levels of soybean solids, addition of 4% sucrose or 0.2% sodium chloride enhanced suspension stability. A good suspension stability required beverage pH to be in the range of 7.4-7.7; pH's above and below this were detrimental to suspension stability.
When the lipid component was studied, finished beverages showed up to 45% bound lipid (percentage of total lipid not extracted by Skelly-solve F). Lipid binding increased with increased pressure and temperature of homogenization.

Total solids suspension stability was enhanced by addition of oil to the soybean beverage. If free lipid was removed from the whole bean or cotyledon before beverage manufacture, suspension instability occurred. Suspension stability was further decreased if bound lipid were removed as well. Priepke (173) claimed that this proved the lipid component to be a prime factor in suspension stability. When he accelerated settling by centrifugation, evidence was found of a protein-lipid complex formed by processing that was less dense than the density of protein alone; therefore suspension was aided.

7. Reconstitutability of dehydrated soybean beverages

Little information is available regarding the rehydration properties of dehydrated beverages containing soybean proteins. Nitrogen or protein dispersibility index is usually used as a measure of protein dispersion of flours, isolates and concentrates. The only soybean beverage powders evaluated for reconstitutability by criteria usually applied to dried milks have been the spray dried cheese whey soy flour mixture and whey soy drink mix (154, 158).

a. Protein dispersibility

Smith et al (97) showed that monovalent salts retarded protein dispersibility of defatted soybean meals; divalent salts of calcium and magnesium had a much greater effect. However, no neutral
salt concentration gave a greater dispersibility than water at the natural pH of the system.

Smith and Circle (96) investigated the effects of pH on protein dispersibility. Best dispersibility was in the alkaline range. When calcium chloride was added to the dispersion medium, the depressing effect of the salt on protein dispersibility was best overcome in alkali, meaning that if hard water is used to disperse defatted meal, the adverse effects can be overcome if alkali is added.

McWatters and Holmes (187) examined the effects of pH and salt concentration on the nitrogen solubility of defatted soy flour. They showed by multiple regression analysis that pH was the primary determinant of nitrogen solubility. They were unable to identify the effects of soluble nitrogen on emulsion capacity and viscosity because of pH effects.

Volkert and Klein (188) examined the relationship between protein dispersibility and emulsion characteristics of a full fat flake, two concentrates (toasted and untoasted) and an isolate. They demonstrated a linear correlation between protein dispersibility index and emulsifying activity and stability. However, pH was the major factor in determining whether an emulsion would form or not.

Smith et al (189) showed that protein dispersibility in water depended largely on crushing the cell structure of the seed and the fracture of any membrane-like structure that might prevent contact of the water with the protein. These authors did not investigate the nature of the undispersed protein.
As soybean meal ages as during storage after processing, protein dispersibility is decreased. Decreases are greatest with storage at room temperature (190). Smith and Circle (96) corroborated these results and reported a decrease of about 1.1% per month in protein dispersibility for the first few months of storage at room temperature.

Fukushima and Van Buren (191) systematically investigated the effects of physical and chemical processing factors on the redispersibility of dried soy milk proteins. These authors showed that heating conditions before drying had the greatest effect on redispersibility of the proteins after drying. Increased alkalinity or low total solids during heating increased redispersibility after drying. Redispersibility was greatly increased by drying the heated proteins from a concentrate of low total solids. The use of emulsifiers affected dispersibility only slightly; dispersibility was maximized at an emulsifier concentration of 0.6% and HLB range 7 to 10. Tween 60 alone (HLB 14) had no effect on protein dispersibility. Protein dispersibility after drying increased markedly with the addition of the disulfide bond splitting reagents sodium bisulfite, cysteine, 2-mercaptoethanol or hydrogen peroxide to the solution before heating and drying. Best redispersibility was found by use of 0.001 M cysteine. The authors concluded from their results that the formation of disulfide bonds during heating and drying were responsible for the insolubilization of the soybean proteins during drying.

The same authors (192) went on to investigate the mechanisms of protein insolubilization during drying of soy milk.
They were able to demonstrate that polymerization took place both through disulfide bond formation and through hydrophobic bond formation. After brief heating, soy milk (7% TS) had $2 \times 10^{-4}$ M active free sulfhydryls exposed; these were involved in polymerization during drying and led to insolubilization of 35% of the total protein. However, these exposed groups were inactivated by prolonged heating before drying so the redispersibility of the dried powder increased.

Amounts of proteins insolubilized through hydrophobic bonds increased evenly with heating time before drying and reached a plateau where 40-50% of the total protein was insolubilized by hydrophobic bonds.

The authors concluded from these results that insolubilization through formation of covalent bonds other than disulfide bonds was remote, because almost all proteins treated with an SH-blocking reagent before drying were solubilized by sodium dodecyl sulfate that does not split covalent bonds.

Nash et al (193) examined the effect of 2-mercapto-ethanol on protein extractability for fresh and aged defatted soybean meals. 0.01 M reductant in the extraction medium increased protein extractability; fractions extracted were mainly 7S and 11S proteins. Analyses of the reductant extracts showed that the 7S and 11S fractions repolymerized partly when the reductant was removed by dialysis except with aged meals. Reductant was not as effective in extracting protein from aged meal.

The same authors (194) also investigated the extraction of protein from steamed defatted meal with 0.01 M 2-mercapto-ethanol. In contrast to unsteamed meal, the 11S fraction did not
re polymerize when dialyzed free of reductant. If the steamed meals were extracted with 0.1 M 2-mercaptoethanol, both fractions lost the ability to re polymerize, in contrast to unsteamed meal. The authors believed that meal aging or mild heating of the meal caused conformational changes in the 7S and 11S proteins that permitted 2-mercaptoethanol to reduce critical internal disulfide bonds which, once broken, allowed structural changes that prevented subsequent re polymerization.

Hand et al (115) reported difficulty in interpreting soluble nitrogen values in pilot plant studies on dry soymilk because, while heating the fluid soymilk, soluble nitrogen content passed through a minimum and increased again with prolonged heating, when measured in the dried product. This result was later explained by Fukushima and Van Buren (192) on the basis of inactivation of exposed SH groups during prolonged heating, resulting in decreased possibilities of insolubilization through S-S polymerization.

Mustakas et al (143) reported that nitrogen solubility index of their beverage base in water was increased from 24.2% to 66.5% by wet milling of the extrusion cooked full fat soy flour used in product manufacture. On the other hand, NSI of the spray dried LPC beverage base was 15.2% with no added fat and 11.2% with added fat (137).

Aminlari et al (174) carried out a detailed study of the effects of processing variables on the protein dispersibility of a spray dried whole soybean milk base. PDI of the spray dried powder was increased from 50 to 57 (14%) by using ammonium bicarbonate
instead of sodium bicarbonate to Blanch the cotyledons. Increase in PDI also occurred when the soybean slurry was homogenized at high pressures and when sodium bisulfite was added to the milk base before spray drying. Increasing pH to 9 before drying also increased PDI. Best PDI obtained was 72%, a value higher than those previously reported for dried soybean beverages; this was undoubtedly due to the treatment with sodium bisulfite, previously shown by Fukushima and Van Buren (191) to increase soybean protein redispersibility by splitting disulfide linkages.

II. Reconstitutability of Dry Powders

Properties related to the reconstitution of dry milks with water have been studied extensively. King (195) has written an excellent review of the available literature and much of the following discussion is taken from his paper.

Although general terms such as solubility or dispersibility may be used to describe the recombination of dry milk with water, strictly speaking, dispersibility, solubility, sinkability and wettability are limited but distinct terms. For example, casein is dispersible but lactose, mineral salts and undenatured whey proteins are soluble. Sinkability refers to the ability of the powder particle to break the surface tension of the water and wettability means penetration of the water into the powder particle (175). All of these functions occur during the process of recombining dry milk with water.

Factors that contribute to the reconstitutability of dry powder may be: 1. the physical properties of size, shape, surface and
density of the dried particle, uniformity, air content, the presence of additives and the composition, especially the solids-not-fat to fat ratio; 2. the chemical properties such as the degree of protein denaturation brought about by heat treatment or storage conditions; 3. the conditions for recombining such as temperature of the water and the powder, the hardness of the water and the time and nature of mixing; 4. processing conditions during manufacture such as type of dryer and atomization system, heat stability of the milk, preheat treatments of milk and concentrate, homogenization pressure, temperature of homogenization, total solids of the mixture homogenized, total solids of the concentrate to be dried, air dryer outlet temperature, contact time, recycling of fines, degree of agglomeration and storage time and temperature.

A. Particle Size

The size of a particle is that dimension which best describes the subdivision of the particle. For a spherical particle, the diameter serves this function. Particles of irregular shape can be characterized in other ways; for example, techniques that measure other size dependent properties such as volume, surface, light scattering or resistance to motion in a gas can be used to yield various equivalent diameters.

The particle size distribution is the frequency of occurrence of particles of every size present. The mean characteristics of a large number of particles is the thing that can be studied statistically. The frequency of occurrence may be reported as the number of particles or as a weight greater than or smaller than a stated size
or range of sizes. Number size distributions are normally obtained by microscopic size measurements whereas weight size distributions are obtained by other methods such as sieving.

Most fine particle systems have particle distributions that follow the logarithmic form of the Gaussian statistical law of errors (log normal distribution function) (196). This means that the two well established parameters of the log-normal law, often evaluated graphically, adequately describe particle size distributions. These parameters are the arithmetic or geometric mean as the average size and the arithmetic or geometric standard deviation as the dispersion or measure of the spread in particle sizes around the average or mean size. The two main graphical presentations are the histogram, in which particle amount by size class is plotted against particle size and the cumulative plot in which the percentage of particles with size less than or greater than the indicated size is plotted against the particle size.

In many cases, if particle size distribution is plotted on log probability graph paper and does not yeild a straight line as should be the case if the distribution is log-normal, a modified log-normal distribution may be obeyed. This interpretation is particularly useful for powders obtained by spray drying. This can mean that size distribution is multimodal (has two or more mean sizes) rather than unimodal as in a log-normal distribution.

Optical microscopy is the most direct method for measuring particle size distributions and many measurements of milk powder particles have been made in this manner. Some measurements of the
particle size distributions of whole milk powders have been made by Hayashi et al (197), Manus and Ashworth (198), Janzen et al (199), Hanrahan et al (200), Buma (201) and Holsinger (202). Not all of these authors gave a mathematical description of the size distribution. However, Hayashi et al reported a log-normal distribution, whereas Holsinger found a modified log-normal distribution of a powder prepared with a 2.5 meter Anhydro spray dryer equipped with a spinning disc atomizer. Buma, on the other hand, reported that his samples could not be described either by a log-normal distribution or by an exponential power law.

Sieving is also frequently used for measuring particle size distribution of milk powders. By using a series of sieves of different size openings, the powder may be separated into a series of fractions by mechanical shaking for a specified time period. However, sieving results are generally not satisfactory because the particles tend to stick together, forming agglomerates with large apparent diameters.

The mean particle size of many powders may also be calculated from their specific surface areas as determined by permeametry, that is, the rate of flow of a gas through a column of packed particles. Fox et al (203) utilized this method to measure specific surface areas of both skim and whole milk powders dried by different methods. Buma (201) also used this method to determine the mean particle size of spray dried skim milk powders.

Sedimentation methods are not suitable for all powders because both particle size and particle density may vary widely within
a powder sample. All measuring techniques using liquids such as the Coulter Counter method of automatic counting involve finding an inert fluid in which none of the powder constituents dissolve during the determination (201).

In spray dried milk powder, individual particles are generally spherical in shape and range in diameter from 10 to 250 micrometers (175). The surface of the spray dried particles is usually smooth; however, wrinkling and roughness increase, the larger the temperature gradient between the hot air and the milk droplets during drying (204). Some agglomeration of particles to form an aggregate may occur in normal spray dried powder, probably due to collision of the partly dried particles shortly after formation. Individual particles may be solid but usually contain one or more air cells formed by entrapment of air bubbles when the filaments formed by the spray nozzles collapse under surface tension forces to form the sphere (205).

Particle size of spray dried products depends on the process variables, especially drying parameters. It has been shown that increasing total solids of the concentrate yields a powder of greater density and with a higher percentage of larger particles (198). Particles also become larger with increasing orifice size and decreasing flow rate pressure (206). Centrifugal spray generally yields a larger particle (175). Hayashi et al (197) showed that particle size of milk powder was not significantly affected by inlet air temperature and preheat treatment but decreased pump pressure or increased total solids yielded a larger particle. Large size particles could be obtained by using low homogenization pressure, low spray pressure
and low spray temperature (175).

Foam spray drying, a one-step method of instantizing carried out by nitrogen injection into the feed line leading to the spray dryer, produced relatively large particles (63-104 micrometers in diameter) that tended to form aggregates (141-429 micrometers in diameter) (200). Buma reported that for whole milk powders, the mean particle size decreased inversely with $\sqrt{p}$ where $p$ equals the spray pressure; orifice diameter seemed to be of minor importance. On the other hand, Masters (207) assessed a $\beta$-function that showed that for dry particle size to remain the same with increasing feed total solids, atomization conditions must be changed so as to obtain a reduction in the wet spray particle size. Inclusion of air during droplet formation in the dryer changed the particle size distribution at low total solids of the feed.

B. Particle Density

Densities of milk powders are classified into 3 groups: bulk or apparent density, particle density and density of the dry milk solids. Of the three, bulk density is of the most practical importance because of its influence on packaging costs.

Bulk density is equivalent to weight per unit volume and is frequently expressed as g/cm$^3$. Manufacturing procedures greatly influence bulk density, mostly by their influence on air content of the powder particles. Foam-spray dried milk has the least bulk density (.3 g/cc), roller dried is next (.3-.5 g/cc) and regular spray dried is highest (.5-.8 g/cc) (175). Increasing total solids of the concentrate, reducing the spray pressure and using a large nozzle orifice
increases bulk density (175). Agglomeration for instantizing reduces bulk density 40-60% (175). The bulk density of foam spray dried milk could be improved by compressing at 500 to 600 psi (35.2 to 42.2 kg/cm$^2$) (208).

Less uniformity in particle size distribution can increase bulk density since voids in the structure of regular particles may be occupied by smaller ones; particle shape as well as size will also affect the closeness of the packing (196).

Particle density and porosity are influenced mainly by entrapped air and can have an important effect on the ability of solvent to penetrate the powder particle during reconstitution. Buma (201) has demonstrated that particle density of spray dried powders increases with decreasing particle size. Viscosity and air incorporation into the concentrate before drying both affect particle density.

True density refers to the air-free solids of the powder. Moisture content and ratio of solids-not-fat to fat are the chief variables affecting true density which ranges from 1.44 to 1.48 g/cc for nonfat dry milk and from 1.26 to 1.32 g/cc for whole milk powder (175). Berlin and Pallansch (209) measured true densities of whole milk powders dried by different methods and showed that all powders measured had true densities averaging 1.30 g/cm$^3$. However, a wide variation in porosity was observed, with conventional spray dried powder being the least porous. Vacuum-shelf foam-dried powder had essentially no micropore structure.
C. Particle Structure

The physical structure of dry milks is defined as the way the chemical components are distributed and interact with one another in the dried powder particle. These interrelationships influence the particle characteristics upon reconstitution.

The physical state of milk constituents in fluid milk may carry over into the dried particle. Therefore, the dry milk particle may consist of a highly concentrated lactose syrup or glass in which the proteins and some mineral salts are colloidally dispersed and fat, if present, emulsified. Part of the lactose may be crystalline, usually the α-hydrate. If fat is present, the size of the fat globules varies considerably. Some fat globules are found at or near the surface of the powder particle. King (210) used fluorescence microscopy to examine milk powder particles. In properly prepared whole milk powder, King reported that the fat globules appeared uniformly distributed in the solids-not-fat phase; however, if manufacture or storage was defective, coalesced fat patches could be seen spread around the air cells and on the periphery of the powder particles. Some small particles were joined by fat bridges. In contrast to spray dried whole milk powders, fat in roller dried whole milk powder appeared as large pools, patches and globules inside and outside of the particles; the fat could be easily extruded from these particles.

Foam dried whole milk powders prepared by the method of Sinnamon et al (211) had the fat evenly distributed as small droplets throughout the "dog-bone" shaped particles (212).
Whether the surface of the powder particle has the same composition as the whole is still not fully resolved.

Ranz and Marshall (213) suggested that evaporation proceeds from a solution during spray drying as though the surface were saturated with solute; this means that there is rapid concentration of solution at the surface of the drying droplet and consequent migration in solution to the surface. The extent of migration of solutes to the surface probably varies with conditions. Pyne (214) showed with contact angle measurements of water droplets on the flat surface, that typical nonfat dry milk (62% lactose, 38% protein) had a particle surface corresponding to 79% lactose and 21% protein. Bockian et al (215) showed that the readily soluble sodium, potassium, chloride and lactose seemed to be preferentially oriented on the surface of instantized nonfat dry milk, whereas normal spray dried milk had a uniform distribution of the components assayed. Holsinger et al (216) demonstrated by a leaching technique that some migration of low molecular weight solutes toward the surface did occur during both spray and foam drying processes. Instantizing apparently increased the amount of osmotically active material at the surfaces of two of the four samples studied.

Washburn (217) apparently first realized the importance of particle size, shape and form in reconstitution. He reported that the finer the particle size the more easily the powder would dissolve up to 75 micrometers; smaller particles were undesirable. An average size of 150 micrometers seemed most desirable. Wilster et al (218) also reported that small powder particles favorably influenced
reconstitutability of dried whole milk.

The principal purpose of agglomeration or instantizing developed by Peebles and Clary (219) is to improve the rate and completeness of reconstitutability of dry milk products. The wettability, sinkability, dispersibility and solubility are all affected. For best agglomeration, the preferred particle range is 25 to 50 micrometers with a minimum of fines below 20 micrometers (175). The instantizing process when applied to dry whole milk improves reconstitutability only slightly because the presence of the fat affects wettability of the powder particles. Processes for vacuum foam dried whole milk powder (211) and foam spray dried whole milk powder (200) were both developed to improve the reconstitutability of dry whole milk. However, in contrast to whole milk powder, the redispersibility of WSDM was not measurably improved by foam spray drying (158) except when the lactose had been hydrolyzed to glucose and galactose (167).

D. Free Fat

The distribution of milk fat bears an important relationship to the rehydration properties of the dried products. Free fat in dehydrated dairy products was originally defined by Holm et al (220) as fat not protected by a protein film or unemulsified fat; it represents that portion of total lipids extractable with nonpolar solvents under standardized conditions. Free fat has been associated both with poor dispersing properties (221, 222, 223) and with lack of oxidative stability during storage (224). Free fat has also been considered to be involved in the formation of the surface scum on reconstituted whole milk (225).
Lowest levels of free fat are found in spray dried powders (3 to 14% of the total fat); roller dried powders contained from 92 to 96% free fat (226). In freeze-dried milk, free fat varied from 58 to 92% (176). Reinke et al (227) showed that free fat in spray dried whole milk could be reduced by lowering the preheat temperature and/or time, homogenizing thoroughly and using a large nozzle orifice at low pressure.

Most authors are agreed that the physical structure of the powder particles is an important factor related to the free fat content. King (210) concluded from his studies that when fat was present in the de-emulsified state, it could coalesce and flow together to form pools of fat that were readily extractable with fat solvents. He further suggested that part of the free fat was on the surface of the powder particles rendering them water repellent. Tamsma et al (228) studied the free fat distribution in vacuum foam dried whole milk prepared by the method of Sinnamon et al (211); they concluded that because free fat increased with decreasing particle size, the free fat was present on the surface of the powder particles, in accordance with King's views. Berlin et al (229) were able to correlate the surface areas of vacuum foam dried whole milks with their free fat content; they concluded that free fat appeared as small globules on the surface of the powder particles.

Buma (230) showed that in spray dried milks, the free fat changed far more than the specific surface area and related the occurrence of free fat to the presence of micropores in the particles (231). In later papers Buma (201, 232, 233, 234, 235, 236, 237, 238)
reported a detailed study of the effects of processing on the free fat content of spray dried whole milks and related the free fat to reconstitutability of the powders as measured by dispersibility and solubility. First, he demonstrated that free fat content was definitely related to particle size; the small particle fractions contained 6 to 10 times as much free fat as the main fraction. He also showed that homogenization of the concentrate before spray drying decreased the free fat content and particle porosity considerably. An increase in particle size also resulted when the total solids of the concentrate to be dried was increased and pump pressure was decreased during drying; Buma believed that this explained the reduction in free fat content reported by Reinke et al (227).

Because porosity was also decreased by homogenization and porosity influences the penetration rate of liquid into the powder particles, the initial rate of dissolution would be affected. Buma found that if the free fat content was 20% or higher, no effect of free fat on dispersibility was observed. Below this value, dispersibility increased with decreasing free fat content. Buma could not identify a relationship between free fat content and solubility however.

On the basis of his results, Buma developed a model for free fat in spray dried whole milk. He proposed that extractable fat consisted of 4 components: (1) surface fat: present as patches on the particle surface, especially in surface folds and at contact points between particles; (2) outer layer fat: fat in the surface layer of the powder particle that can be reached directly by fat solvents;
(3) capillary fat: fat globules inside the particles that can be reached by solvents via capillary pores or cracks; (4) dissolution or "second echelon" fat: fat globules in the interior of the particle that can be reached by solvents through holes left by dissolved fat globules in the outer particle layer. A model is shown in Figure 3.

Although the distinction among forms of extractable fat is empirical, its necessity is explained by the fact that the amount of free or extractable fat varies considerably with time of exposure to the solvent. Whereas surface fat can be extracted with only a short exposure time, there is a large increase with time in the amount of fat extracted from particles with low porosity; this is probably due to dissolution of fat globules in the interior through holes left by dissolving the fat in the outer layers. This process is slow compared to the penetration of fat solvents into the interior of the powder particles through cracks and capillaries. If the powder is very porous, the bulk of the fat can be extracted in a very short time.

Buma's model is useful for considering the physical structure of the powder particles and emphasizes the spatial distribution of the fat globules, the particle size and the particle porosity as the dominant features. The author has concluded that all processing steps before spraying except homogenization have little effect on free fat content in the dry powder. Dispersibility and wettability can only be influenced by the extractable fat located in or on the particle surface.
Schematic of 4 forms of extractable fat in a powder particle. Dark areas represent extractable fat.

1. surface fat
2. outer layer fat
3. capillary fat
4. "second echelon" fat

Buma (238)

Figure 3
DeVilder et al (239) repeated much of Buma's work; however, in contrast to Buma, they demonstrated that free fat content of the powder was significantly increased by heating of the concentrate especially with increasing total solids of the concentrate. The authors explained that the higher temperature of the feed concentrate caused a higher rate of evaporation upon atomization, resulting in increased permeability (more micropores), and therefore increased free fat content.

Hanrahan et al (200) observed that with foam-spray drying, free fat in the powder increased sharply with an increase in the amount of nitrogen injected into the concentrate, in spite of the fact that foam spray drying yielded particles of larger average diameter than spray dried particles. Blaauw and Mol (240) observed that foam-spray drying appeared to reduce wettability even though dispersibility was excellent; this could be partly explained by the increase in free fat content.

Buma's model could explain the increase in free fat observed with foam spray drying. Berlin and Pallansch (209) showed that powders dried by this technique had a large network of micropores; therefore the fat should be more accessible to the solvent if Buma's model is correct. Conventionally spray dried powders were much less porous and what pores there were had much smaller radii; free fat in these powders was very low, because penetration of the solvent into the interior of the particles was limited.

Analysis of free fat extracted from spray dried whole milk showed that it contained more neutral glycerides; saturated fats of
and less mono- or diglycerides, phospholipids and unsaturated fat \( \text{C}_{18} \) than total milk fat \( \text{C}_{18} \).

Storage conditions may have an important influence on the free fat content of whole milk powders. For example, if the lactose crystallizes as a result of moisture uptake during storage, the free fat content of the powder increases sharply. Lampitt and Bushill (226) observed that the fat in spray dried whole milk could be quantitatively extracted as a result of lactose crystallization. King (210) has suggested that the interior of the powder particle is made more accessible to solvent as a result of lactose crystallization. It has also been suggested that the sharp lactose crystals may rupture the fat globule membrane during formation, resulting in an increase in free fat (175).

Guy et al (154) examined the effects of homogenization pressure and method of drying on the free fat content of their cheese-whey soy-flour mixtures. As with whole milk powder, lowest levels of free fat were found in the spray dried product. Free fat content was also reduced by increased homogenization pressure. However, the simple mixing of soy flour with sweet cheese whey (1:2) followed by vacuum shelf drying was sufficient to protect the lipids of soy flour from solvent extraction. The authors explained this effect by the presence of an interfacially active material present in the whey.

E. Wettability

On reconstitution, the surface presented to the water is that of a mass of powder, not the surfaces of the individual particles. The surface is a composite of the particles' surfaces and that of the
air in the voids at the powder-water interface. Therefore, wettability may be considered to be the ease with which the powder particles make contact with water which in turn depends on the properties of their surfaces. The tendency of dry milk to form lumps and float on the water surface is generally considered to indicate poor wettability (242).

1. Effects of particle size and shape on wettability

Small particle size and symmetrical shape enhance close packing and inhibit penetration of the water. Larger particles more irregular in shape have more spaces in the interstices for wetting. Increased particle size by aggregate formation and larger air spaces between agglomerates also favor wetting (215). Bockian et al (215) also believed that preferential orientation of soluble constituents toward the powder particle surface as a result of instantizing improved wettability. Peebles (243) agglomerated NDM to form aggregates and evaluated the capillarity of the resulting powder. He concluded that capillarity caused water to penetrate the agglomerated powder rapidly and penetration was not blocked by particle swelling. Pyne (214) demonstrated that the interspaces of the powder mass could act as capillaries and that NDM reconstituted at a rate governed by the capillary characteristics of the powders. The larger the particles, the greater the hydraulic radius of the interspaces and the larger the hydraulic radius the more rapid the movement of water into the capillary will be and the lower the rate of increase of TS concentration in the reconstitution.
Pyne (214) also measured contact angles of water droplets placed on the surface of sieved fractions of NDM. Surfaces composed of particles with surface average diameters below 9 micrometers were not wettable. With surfaces above 10 micrometers, wettability increased rapidly; above 40 micrometers no contact angle was apparent because the water droplet disappeared into the powder mass.

Pyne (214) also examined dry whole milk at room temperature and found that surfaces of particles with a surface-average diameter below 60 micrometers were nonwettable.

2. Effects of powder composition on wettability

a. Milk fat

Coulter (cited by King, 195) had already pointed out that the amount and dispersion of the fat affected wettability. Powders containing 5 to 15% fat showed comparable rates of wetting; powders with fat contents ranging from 18 to 32% also showed comparable wettability but wetted more slowly than powders with lower fat levels.

The physical state of the lipid phase has also been related to wettability. Powders in which the fat phase is present as a liquid are more readily wetted than if partial or complete solidification of the fat has occurred (244, 245). Winder and Bullock (246) demonstrated that wettability was improved by rapid cooling of the powder after drying. Baker et al (247) prepared dry milks containing milk fat fractions of different melting point; powder containing fat of melting point 19-21°C had about the same wettability as NDM. As the melting point increased, wettability decreased.
Baker and Samuels (248) reported that the low-melting fat fraction had lower interfacial tension towards water than the higher melting fractions; however, alterations in interfacial tension with surfactants did not have a marked effect on wettability. It was concluded that the combination of the two factors, physical state and interfacial tension, was important.

When simple triglycerides were homogenized into skim milk and the mixtures freeze-dried, wettability decreased with increasing molecular weight of the triglycerides (249).

Tamsma and Kuntson (250) prepared foam spray dried whole milk powder with improved wettability as measured by sinkability by using liquid fat (melting point 20°C) recombined with skim milk.

Stone et al (221) found that milk powders in which milk fat was replaced by corn oil had better self dispersion at temperatures below the melting point of milkfat.

Ashworth (222) and Mol and DeVries (223) both demonstrated an inverse correlation between wettability and free fat content.

The reconstitution of whole milk powder is further complicated by scum formation attributed to insoluble complexes of fat and denatured protein (225). Leach et al (251) isolated the film formed at the air-water interface on reconstitution of whole milk powder and showed it to be a protein-carbohydrate-lipid complex dependent on the physical state of the lipid phase. Any method to liquefy the lipid weakened the surface film to the point where the milk powder became sufficiently wettable to show self-dispersing properties.
b. Protein

It has been suggested that the physical state of casein limits the rate of dispersion of dried milk (252). Wettability was improved by treatment of the milk with proteolytic enzymes before drying; it was suggested that the casein micelle surface was rendered more hydrophilic by this treatment.

c. Lactose

There is no evidence that lactose crystallization alone influences wettability of the particle surface (253). However, lactose crystallization can occur depending on whether it was initiated in the condensed milk before spraying or developed during storage. The centers of some of the larger particles could contain enough moisture to induce partial crystallization even though the average moisture content is well below that required for crystallization (254). When crystallization occurs, fine cracks appear adjacent to the crystal surfaces; this makes the particles more permeable to gases and fat solvents (210).

d. Moisture content

Ashworth and Gundhardt (255) reported that maximum wettability of dried milk was attained when the total moisture content was about 3.5%. If moisture content was below 2%, powders were less soluble; decrease in wettability occurred during storage if the moisture content was above 4%.
3. Processing effects on wettability

a. Forewarming and condensing

For good keeping quality of dried whole milk, the milk must be forewarmed; optimum time-temperature is 76.7°F for 20 minutes. However, preheating had little effect on wettability (195). On the other hand, heat treatment after condensing reduced wettability. Wettability is poor if the preheated milk is not condensed. Optimum total solids seemed to be between 30 and 40% (198, 252). An increase in wettability with increasing condensation was reported by deVleeschauwer and Van Puyvelde (256) and Mol and de Vries (223) for spray dried skim milk powders.

b. Homogenization

Ashworth (cited by King, 195) considered that homogenization of precondensed whole milk was most important for achieving good wettability. Homogenization was most efficient at fat contents of 1% to 2.5% in the original milk; above 3.5% fat, it was difficult to homogenize efficiently enough to avoid formation of free fat in the powder. With poor homogenization, free fat coated the surfaces of the dried particles, thereby reducing wettability.

c. Spray drying

Ashworth (cited by King, 195) reported that variation of inlet air temperature between 104.4°F and 160°F had no effect on wettability; variation of outlet air temperature between 60°C and 82.2°C likewise had no effect.
d. Storage conditions

Ashworth (cited by King, 195) reported that storage temperature was the most important factor affecting wettability of dried whole milk. This has since been shown to be due to the physical state of the lipid phase, discussed previously.

e. Surfactants

A marked increase in wettability was observed in NDM containing Tween 20. This effect was ascribed to reduction in surface tension of the water, thus aiding penetration into the voids surrounding the powder particles or to orientation of these materials on the surface of the powder, attracting water into the powder (257).

F. Sinkability

Sinkability is closely related to wettability and refers to the rate at which dried milk sinks into quiescent water; it is frequently used as the test for wettability. Sinkability is reported as the percentage of a weighed amount of powder that sank through a known volume of water.

The amount of occluded air in the powder particle has a pronounced effect on sinkability. As particle density is decreased due to gas entrapment as during foam spray drying of skim milk, a point is reached where sinkability is lost and the particles float on the surface of the water. However, foam-spray dried powders that sink like conventionally instantized spray dried powders are all partially aggregated; the sinking property is lost by mechanically breaking up the aggregates by grinding. Therefore, for good sinking properties foam-spray dried powders must be partially aggregated (258). Tamsma
and Kontson (250) increased sinkability of foam-spray dried whole milk powders from 26 to 94% by substituting the low melting fat fraction.

Sinkability is improved by agglomeration brought about by the instantizing process because of increased particle weight, enabling the particles to penetrate the water surface more readily (175).

The sinkability of freshly prepared dry whole milk decreases with increasing storage time (measured in days), coinciding with crystallization of the fat phase (247, 259). Tamsma and Kontson (250) demonstrated this effect with foam spray dried whole milk powder; from a sinkability of 99% initially, sinkability decreased to 26% after 7 days storage at 27°C. Samples prepared with liquid milk fat decreased from 99 to 94%. Sinkability decreased only slightly, however, in powders held at 4°C for 7 days.

Nelson and Winder (260) showed that sinkability of whole milk powder at 25°C could be considerably improved by the addition of surface-active agents such as Tween 60, Tween 80 or lecithin to the whole milk or condensed whole milk before drying. However, at higher concentrations of surfactant, partial churning of the fat occurred during reconstitution. Best sinkability was obtained with a surfactant mixture of HLB 8; almost no churning was observed with this additive.

Guy et al (154) did not report sinkability values for their spray dried cheese whey-soy flour mixtures. However, Holsinger et al (158) reported a sinkability value of 69.7% for unfortified whey soy drink mix. When vitamins and minerals were added, sinkability
increased to 81.8%.

The substitution of a beef tallow oil fraction for soybean oil in the whey soy drink formulation significantly increased the sinkability of the spray dried powder, even though the densities of the oils used in the formulations were virtually identical. These results suggested that the structure of the dried particles was different; the beef tallow oil fraction apparently made the particles more wettable thereby enhancing sinkability (166).

G. Dispersibility

Dispersibility of a powder may be considered to be a measure of the rate-of-solubility of that powder, that is, the ability of the powder mass to disintegrate into the solvent after penetration of the solvent into the powder (253). Small particles, once dispersed, may dissolve faster than large ones; however, undissolved but dispersed particles could persist for some time in a fluid product reconstituted from large particles.

The quality of self-dispersion is the ability of the powder to disperse rapidly and completely into the solvent without the use of mechanical stirring (221). In order to do this, the powder must first have the ability to penetrate the solvent surface; therefore good wettability is the prerequisite for good dispersibility.

Dispersing properties of dried milks are influenced by three factors: 1. the physical structure and chemical composition of the powder, 2. manufacturing and storage conditions, 3. the reconstitution procedure (195).
l. Effect of composition on dispersibility

a. Milk fat

As the fat content of dried milk increases, self dispersion decreases (221). However, a wide variation in the values for self dispersion of commercial whole milk powders suggested that other factors were involved as well. Composition of the lipid phase also affects dispersibility; dispersibility of the powder decreased with increasing melting point of the milk fat fraction (247). The powder containing the fraction melting at 19-21°C had about the same dispersibility as nonfat dry milk. Stone et al (221) showed that powders containing corn oil in place of milk fat had higher self-dispersion values at temperatures below the melting point of milk fat.

Changes in dispersibility have been related to the free fat content of the milk powder. Janzen et al (199) found that free fat on the surfaces of whole milk powder particles caused the particles to adhere to the walls of the air elutriation apparatus used to size the particles. After the powders were washed with a fat solvent like carbon tetrachloride, fractionation became possible. In addition, the powders were more dispersible. Tamsma et al (228) reported that the dispersibility of foam dried whole milk, prepared by the method of Sinnamon et al (211), decreased with increased free fat content. On the other hand foam spray drying considerably improved the dispersibility of whole milk powder, even though free fat also increased (200).
b. Milk protein

The physical state of casein has an important effect on the rate of dispersion of dried milk. An important consideration is the total heat treatment applied during processing. For example, roller drying exposes the film of milk to severe heat with the result that the dried powder contains a high percentage of denatured casein (175). Although spray dried milk usually has very little denatured casein, denaturation will increase by overheating the condensed milk or exposing the milk particles to excessive heat in the dryer (175). Under normal reconstitution procedures, severely denatured casein will not form a stable dispersion.

2. Effects of processing methods on dispersibility

The chief processing method for producing self dispersing dried milk for beverage purposes is spray drying followed by instantizing. Other drying methods have also been developed to yield dry milks with this desirable property. The various processing steps involved have varying effects on the dispersibility of the finished powder.

a. Forewarming and condensing

Ashworth (cited by King, 195) demonstrated that the high preheat treatment necessary for good keeping quality of dry whole milk had little effect on dispersibility as expressed by wettability. However, Reinke et al (227) showed that self dispersion properties were reduced by high preheat temperatures. Heat treatment after condensing also had an adverse effect.
b. Homogenization

Reinke et al (227) observed that the self dispersion of dried whole milk was reduced if the milk was homogenized before condensing or homogenized after condensing. Double homogenization increased this effect.

c. Drying parameters

Coulter (261) and Pyne (214) first demonstrated that regulation of the amount and type of gas in the feed line of a conventional spray dryer would improve the reconstitution characteristics of whole and skim milk powders. However, such powders still did not reconstitute as easily as commercial powders instantized by rewetting, agglomerating and redrying.

Bockian et al (215) attributed the good dispersibility of "instant" milk powders to the migration of osmotically active constituents to the surface of the rewetted and redried particles. However, Holsinger et al (216) were unable to relate dispersibility to the spatial distribution of the milk constituents within the powder particles.

Mather and Hollender (262) reported a significant increase in the self-dispersion properties of dried whole milk prepared with the addition of surface-active agents. Use of Tween 80 (polyoxyethylene sorbitan mono-oleate) yielded a readily dispersible powder. However, if mechanical agitation was employed, the fat churned during reconstitution. The more hydrophilic surface active agents enhanced dispersibility, but promoted churning. The maximum concentration of hydrophilic agent that could be used without churning
was 0.06%.

Hollender (263) also reported an increase in self dispersion of whole milk powders prepared with surface active agents stored at or near the melting point of the milk fat. He attributed this effect to the migration of the surface active agents to the surfaces of the powder particles.

Dispersibility decreases with increasing bulk density in both nonfat dry milks (253) and in whole milk powders (264). Sinnamon et al (211) also reported a decrease in dispersibility with increased bulk density of vacuum foam dried whole milk. In a previous study Harper et al (265) had suggested that good self-dispersing properties would not be found in dried milks of bulk density greater than 0.4g/cc because of high concentrations of solutes in the reconstituting liquid.

Lascelles and Baldwin (266) studied the effect of the presence of fines in agglomerated whole milk powder on dispersibility. Best dispersibility was achieved when the percentage of fines less than 90 micrometers in diameter in the powder was below 20% by weight of fines. As the percentage of fines increased, dispersibility sharply decreased.

The injection of gas into the preconcentrate under pressure substantially improved the dispersibility of dried whole milk in spite of increased levels of free fat (200). When skim milk was dried by this method, dispersibility was almost independent of total solids in the concentrate, provided the viscosity of the concentrate was low before drying (155). A concentrate of viscosity
>5000 centipoise yielded a powder of low dispersibility probably because of agglomeration of the casein as a result of the high viscosity.

Tamsma et al (258) prepared skim milk powders with good dispersibility by low foaming to produce a higher bulk density. Although bulk density did not exceed 0.4g/cc, foaming with CO2 as compared to N2 gas reduced dispersibility of whole milk powder (250).

Kontson et al (267) found that foam spray dried whole milk had a wide diversity in particle size as measured by sieving. Contrary to findings in studies of instantized nonfat dry milk, the large sized particles (>710 micrometers in diameter) had the poorest dispersibility. These particles comprised 10 to 35% of the powders and consisted of fused aggregates of foamed particles. The authors found a linear relationship between the proportion of these large particles in the powder and dispersibility; dispersibility decreased by 4% for every 10% of the coarse fraction present in the powder. However, when the coarse fraction was crushed and recombined with the other fractions, dispersibility increased by 6 percentage points (268).

The vacuum foam drying technique developed by Sinnamon et al (211) was specifically aimed at achieving a readily dispersible whole milk powder. Eskew et al (269) studied the dispersibility of the foamed powders in detail. The authors demonstrated that forewarming the milk to 37.8°C for 2, 5 and 3.5 minutes had no effect on initial dispersibility. However, after storage for 1 month at 37.8°C, dispersibility varied, with best dispersibility observed in
milk given the shorter heat treatments. Foam dried whole milk could be stored for 1 year at 22.8°C with no change in dispersibility; however, some loss occurred during storage at 37.8°C. This change might be due to an increase in free fat during storage at the higher temperature as has been observed in studies with spray dried whole milk (225).

3. Effect of reconstitution method on dispersibility

The greatest factors influencing the extent and speed of dispersion of dried milk in water are the type and vigor of stirring and the temperature of the water and powder. For example, even if a powder will not sink, suggesting poor wettability, it may still have excellent dispersibility even in ice water as soon as some external agitation is applied.

a. Stirring

For home use, milk powders that require minimum amounts of energy to reconstitute are the most satisfactory.

Harper et al (265) mixed dried milk with sand and pulled water through the mixture under vacuum. This procedure was considered to be mechanically equivalent to dispersion of milk solids throughout water which has been stirred. Use of increased amounts of sand to disperse the same amount of powder was equivalent to increasing stirring speed. Their findings indicated that speed of stirring is far more effective in dispersing milk powder than duration. However, Sinnamon et al (211) demonstrated that dispersibility of vacuum foam dried whole milk powder increased with increased stirring time when the powder was reconstituted in ice water while
stirring with a teaspoon.

Kontson et al (267) showed that even though the coarse particle fraction of foam-spray dried whole milk powder was less dispersible than the smaller particle fractions by the test used, if agitation time was increased, the fraction was completely dispersible.

b. Effect of water temperature

The water temperature is most important for the satisfactory reconstitution of whole milk powder.

For spray dried whole milk, best reconstitution was found at 43.3°C whereas at 4.4°C, reconstitution was poor (218, 270). For skim milk powder, Troesch and Wilk (271) reported a maximum dispersibility at about 60°C. Aldrich and Downs (272) reported a decrease in powder dispersibility at water temperatures between 50 and 100°C for skim milk powder, instant skim powder and dried whole milk.

Stone et al (221) reported that water temperature between 1.7 and 32.2°C had little effect on the self dispersion of spray dried whole milk; however, between 35°C and 37.3°C, near the melting point of milk fat, a sharp increase in self dispersion occurred followed by a gradual increase as water temperature increased from 37.3°C to 60°C. Spray dried skim milk increased in dispersibility gradually over the temperature range; at 65.6°C, a value close to that found for dried whole milk at 37.8°C was achieved.

Stone et al (221) also reported that if water temperature was held constant at 23.9°C and the temperature of the
powder was increased, whole milk powder again showed a sharp increase in self dispersion in the region of the melting point of the milk fat. If the dried whole milk was cooled slowly from 48.9°C to 24.4°C self dispersion decreased; however, these values were different from the initial value at 22.2°C indicating a different physical state of the freshly solidified milk fat. When corn oil was substituted for milk fat, there was a gradual increase in self dispersion with increasing powder temperature; the dispersibility was also greater at powder temperatures below the melting point of milk fat, probably because the oil was liquid at these temperatures.

Lascelles and Baldwin (266) reported that the dispersibility of agglomerated whole milk powder was poor at reconstitution temperatures below 45°C, because powder wettability was low. They attributed this to lack of melting of fat in the powder. A reconstitution temperature between 55 and 65°C was optimal.

Sinnamon et al (211) reported that a major advantage of vacuum foam dried whole milk was that it was readily disperisible at 38°F (3.3°C) when stirred with a teaspoon; this was not necessary because the powder would self-disperse completely in cold water within 4 minutes. On the other hand, dispersibility of foam-spray dried whole milk powder in ice water varied with nitrogen injection rate during product manufacture (200).

Guy et al (154) did not report dispersibility values for their spray dried cheese whey-soy flour mixtures. However, Holsinger et al (158) reported a dispersibility of 89.3% for unfortified whey soy drink mix when dispersed in water at 5°C. Dispersibility
decreased to 81.7% in the product fortified with vitamins and minerals, probably due to the presence of calcium in the mineral mix. However, both dispersibilities are equivalent to those reported by Tamsma and Sutton (264) for spray dried whole milk powders.

H. Solubility

Solubility and dispersibility are terms frequently used interchangeably when referring to the reconstitutability of dry milks. Although only the lactose and mineral salts may be considered truly soluble, ultimate "solution" of any fresh powder depends on the extent of casein destabilization during processing (253). This means that solubility is empirical and will vary with the test used. The ones most commonly employed are those of the ADM (273, 274) in which the volume of washed sediment is measured after mixing and centrifuging under specified conditions.

The temperature-time relationship of preheating the milk is directly related to the solubility of the resulting powder. Total solids of the concentrate during heating is also related to powder solubility (198, 275). Although it seems likely that preheating fluid milk within the normal ranges of time and temperature used would have much effect on the solubility of the dried powder, the extent of heat treatment could influence heat coagulation of the protein during drying (275) just as it affects coagulation of evaporated milk during sterilization (276). Small particle size, small uniform sized fat globules and care not to overheat the powder during drying led to good solubility for whole milk powders (218). Manus and Ashworth (198) found a direct relationship between
solubility and homogenization; more concentrated milk was homogenized more efficiently and yielded a more soluble powder.

The degree to which casein rehydrates during reconstitution depends not only on the characteristics of the individual powder but on the speed and duration of stirring and the temperature of the powder-water mixture (277, 278). The speed of stirring had a far greater influence than time of stirring. Harper et al (265) confirmed the findings of Howat et al (278). However, they suggested that the benefits of more rapid stirring were related to a more rapid reduction of the solids concentration close to the dissolving powder particles. High local concentrations of milk salts and lactose in the aqueous phase in contact with the dissolving particle rendered part of the protein insoluble. Therefore, for good self dispersion, milk powders must have a bulk density $< .4 \text{ g/cm}^3$.

Howat and Wright (277) demonstrated that solubility of roller dried skim and whole milks increased with increase in water temperature to 50°C; above 50°C, solubility decreased, probably due to precipitation of whey proteins and casein. However, with spray dried powders, this effect was not observed.

Tamsma and Sutton (264) examined the effect of bulk density of conventionally spray dried whole milk powders on solubility. Powders were prepared with bulk densities of .25, .4 and .6 g/cc. Solubility index increased from 0.1 ml to 1.6 ml with increased bulk density in spite of low dryer air temperature (121°C).

Tamsma and Kontson (250) also showed that the type of gas used to prepare foam spray dried whole milk powder of bulk density
.4 g/cm² affected solubility. Powders foamed with CO₂ during manufacture were less soluble than those foamed with nitrogen. Substitution of a low melting milk fat fraction or lowering of the fat content improved solubility, however. Foaming of whole milk powders also improved solubility over those conventionally spray dried (268).

III. Cheese Whey

Cheese whey is the greenish-yellow serum of milk resulting from the removal of fat and casein from whole milk during cheese manufacture. It contains roughly 50% of the milk solids and most of the water soluble vitamins and minerals (279).

The economic disposal of cheese whey has become a growing problem in the United States mainly due to stringent antipollution regulations. For every 10 pounds (4.54 kg) of cheese produced, 90 pounds (40.9 kg) of fluid whey are obtained. In 1978, 31.7 billion pounds (14.41 billion kg) of sweet cheese whey were produced in the United States; in addition, 6.2 billion pounds (2.82 billion kg) of acid cheese whey were produced as the byproduct of cottage cheese manufacture (280).

The major problem encountered in whey utilization is related to its low total solids concentration and furthermore in some ways, whey may be viewed as a dilute solution of lactose. The composition of sweet and acid whey solids is shown in Table 9 (281). Although the carbohydrate content appears the same, dehydration of acid whey has presented special problems because of the presence of about 10% lactic acid as part of the carbohydrate content; sweet whey solids
Table 9. Composition of Cheese Whey Solids

<table>
<thead>
<tr>
<th></th>
<th>Sweet Whey</th>
<th>Acid Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (Total N x 6.38)</td>
<td>12.93</td>
<td>11.73</td>
</tr>
<tr>
<td>Milkfat</td>
<td>1.07</td>
<td>0.54</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>74.46</td>
<td>73.45</td>
</tr>
<tr>
<td>Ash</td>
<td>8.35</td>
<td>10.77</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.19</td>
<td>3.51</td>
</tr>
</tbody>
</table>

Posati and Orr (281)
contain less than 1% lactic acid (282).

A. Whey Proteins

The total solids contain anywhere from 11.1 to 16.6% protein in sweet whey and 8.0 to 12.6% protein in acid whey (283). The amino acid patterns for both sweet and acid whey solids are shown in Table 10.

The whey proteins are comprised of five major fractions, listed in Table 11 (284). Although concentrations vary in the milk of different breeds, β-lactoglobulin is always the major whey protein. Bovine serum albumin (BSA) has been shown to be identical with the crystalline albumin obtained from bovine blood serum (285). The immune globulins are present in the lacteal secretions for passive immunization of the calf (286). α-Lactalbumin is one of the two proteins comprising the lactose synthetase enzyme, the enzyme responsible for the biosynthesis of lactose (287). The proteose-peptone fraction is a mixture of heat stable phosphoglycoproteins insoluble in 12% trichloroacetic acid (288).

Some properties of the individual major whey proteins are listed in Table 12 (289).

Average amino acid composition of the individual major whey proteins are listed in Table 13. All three are very high in lysine and in addition, both α-lactalbumin and BSA contain appreciable levels of cystine. β-Lactoglobulin represents a particular good source of methionine.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Sweet Acid g/100g sample</th>
<th>Acid Acid g/100g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>1.030</td>
<td>1.008</td>
</tr>
<tr>
<td>His</td>
<td>0.230</td>
<td>0.230</td>
</tr>
<tr>
<td>Arg</td>
<td>0.375</td>
<td>0.327</td>
</tr>
<tr>
<td>Asp</td>
<td>1.269</td>
<td>1.149</td>
</tr>
<tr>
<td>Thr</td>
<td>0.817</td>
<td>0.590</td>
</tr>
<tr>
<td>Ser</td>
<td>0.622</td>
<td>0.541</td>
</tr>
<tr>
<td>Glu</td>
<td>2.248</td>
<td>2.096</td>
</tr>
<tr>
<td>Pro</td>
<td>0.786</td>
<td>0.699</td>
</tr>
<tr>
<td>Gly</td>
<td>0.280</td>
<td>0.211</td>
</tr>
<tr>
<td>Ala</td>
<td>0.598</td>
<td>0.506</td>
</tr>
<tr>
<td>Cys</td>
<td>0.253</td>
<td>0.211</td>
</tr>
<tr>
<td>Val</td>
<td>0.697</td>
<td>0.579</td>
</tr>
<tr>
<td>Met</td>
<td>0.241</td>
<td>0.221</td>
</tr>
<tr>
<td>Ileu</td>
<td>0.719</td>
<td>0.581</td>
</tr>
<tr>
<td>Leu</td>
<td>1.186</td>
<td>1.116</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.363</td>
<td>0.300</td>
</tr>
<tr>
<td>Phe</td>
<td>0.407</td>
<td>0.386</td>
</tr>
<tr>
<td>Trp</td>
<td>0.205</td>
<td>0.241</td>
</tr>
</tbody>
</table>

Posati and Orr (281)
Table 11. Distribution of the Whey Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactoglobulin</td>
<td>46.9</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>17.2</td>
</tr>
<tr>
<td>bovine serum albumin</td>
<td>5.5</td>
</tr>
<tr>
<td>Immune globulins</td>
<td>12.5</td>
</tr>
<tr>
<td>Proteose-peptones</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Calculated from Jenness and Patton (284)
Table 12. Some Properties of the Major Whey Proteins

<table>
<thead>
<tr>
<th></th>
<th>β-lactoglobulin</th>
<th>α-lactalbumin</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Wt</td>
<td>35,500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16,000</td>
<td>65,000</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>5.18</td>
<td>4.1-4.8</td>
<td>4.75</td>
</tr>
<tr>
<td>Mobility x 10&lt;sup&gt;5&lt;/sup&gt; at pH 8.4, μ=0.1</td>
<td>-5.1</td>
<td>-4.2</td>
<td>-6.7</td>
</tr>
<tr>
<td>E&lt;sub&gt;1% 1 cm at 280&lt;/sub&gt;</td>
<td>9.3</td>
<td>--</td>
<td>6.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>β-lactoglobulin undergoes monomer ↔ dimer and dimer ↔ octomer association-dissociation reactions under the proper conditions (Townend and Timasheff, 289)
Table 13. Average Amino Acid Composition of the Major Whey Proteins

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>ß-lactoglobulin</th>
<th>ß-lactalbumin</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>11.4</td>
<td>11.5</td>
<td>12.8</td>
</tr>
<tr>
<td>His</td>
<td>1.6</td>
<td>2.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Arg</td>
<td>2.9</td>
<td>1.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Asp</td>
<td>11.4</td>
<td>18.7</td>
<td>10.9</td>
</tr>
<tr>
<td>Thr</td>
<td>5.8</td>
<td>5.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Ser</td>
<td>5.0</td>
<td>4.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Glu</td>
<td>19.5</td>
<td>12.9</td>
<td>16.5</td>
</tr>
<tr>
<td>Pro</td>
<td>4.1</td>
<td>1.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Gly</td>
<td>1.4</td>
<td>3.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Ala</td>
<td>7.4</td>
<td>2.1</td>
<td>6.2</td>
</tr>
<tr>
<td>(Cystine)</td>
<td>(2.3)</td>
<td>(6.4)</td>
<td>(5.7)</td>
</tr>
<tr>
<td>(Cysteine)</td>
<td>(1.1)</td>
<td>(0.0)</td>
<td>(0.3)</td>
</tr>
<tr>
<td>Val</td>
<td>5.8</td>
<td>4.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Met</td>
<td>3.2</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Ileu</td>
<td>6.1</td>
<td>6.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Leu</td>
<td>15.6</td>
<td>11.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.8</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Phe</td>
<td>3.5</td>
<td>4.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Trp</td>
<td>1.9</td>
<td>7.0</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Jenness and Patton (284)
1. Nutritive value of whey proteins

Whey proteins are of special interest when utilization of whey solids is considered because their nutritional value has been repeatedly demonstrated to be superior to most other proteins in animal nutrition (290, 291, 292, 293, 294, 295, 296). As seen from Tables 10 and 13 whey proteins represent an especially rich source of lysine and sulfur containing amino acids; unlike most vegetable proteins, the limiting amino acids of whey proteins are phenylalanine and tyrosine (297).

The use of whey proteins in human nutrition has been limited because of the low protein content of fluid whey. However, with the development of fractionation methods to separate whey proteins from low molecular weight components, utilization of whey protein concentrates as food ingredients has come closer to reality. Fractionation methods range from simple precipitation procedures such as heat coagulation (298, 299) or treatment with polyphosphates (300, 301) or other protein precipitants (302, 303, 304, 305) to the use of sophisticated techniques such as electrodialysis, gel permeation, reverse osmosis and ultrafiltration, either singly or in combination (306, 307, 308, 309, 310, 311, 312, 313). Concentrates containing from 35 to 90% protein may be prepared by means of the above techniques.

Due to the high lactose content, use of whole whey or whole whey powder as a nutritional supplement for foods has been limited although it is used in many foods, especially baked goods, because of its functional characteristics (314).
Riggs et al (315) evaluated the nutritive value of spray and roller dried whey powders with rats. They also examined a partially delactosed whey powder. They demonstrated that roller dried whey powder did not promote growth until supplemented with lysine, thus showing the inactivation of lysine during processing. Spray dried whey powders all contained protein of better quality than roller dried powders and the protein was more digestible. Delactosed whey was superior to the roller and spray dried wheys in promoting growth.

DeBaun and Connors (316) examined the relationship between in vitro enzymatic digestibility and the in vivo protein evaluation of whey powders. They demonstrated a linear relationship between the enzymatic release of lysine and the biological value of whey powders. These results also showed that roller dried powders contained less lysine and were considerably lower in biological value than spray dried powders. Histidine and tryptophan were also reduced in roller dried powders.

These results demonstrate that if whey powders are to be used as nutritional supplements in human foods, spray dried powders would supply protein of the best nutritional quality.

Vaughan (317) considered the nutritional aspects of whey as a food and compared it to nonfat dry milk as an ingredient in many protein enriched mixtures proposed for supplementation of low-protein indigenous diets in underdeveloped areas. Many of the mixtures such as corn soy milk (CSM) or Pro Nutro contain from 5 to 10% of nonfat dry milk as a protein source. The substitution of whey for nonfat
dry milk in the above-mentioned products reduced the protein and increased the lactose contents somewhat. However, even with the substitution of whey solids, both products were still good protein sources although the levels of the sulfur containing amino acids were low and lysine, threonine and tryptophan were marginal. This problem was readily overcome by using a whey protein concentrate with a protein level similar to that of nonfat dry milk.

Womack and Vaughan (318) made an extensive study of the nutritional value of supplementing corn, rice and wheat with dried whey, a mixture of two-thirds dried whey to one-third full fat soy flour, or a whey protein concentrate prepared by reverse osmosis. Fifty percent of the protein in the corn and rice diets and 40% in the wheat diet was supplied by the supplement. All supplements significantly improved the nutritional quality of grain protein. There was no difference in growth when diets containing dried whey or soy-whey were fed, even though animals fed the dried whey-containing diet developed diarrhea and enlarged ceca. Best growth response was obtained with the diet containing a whey protein concentrate prepared by reverse osmosis.

Forsum et al (319) demonstrated that the best supplement for wheat protein was a whey protein concentrate prepared by gel filtration; nonfat dry milk, fish protein concentrate and lysine monohydrochloride were the other supplements studied. The limiting amino acids in a diet containing 92% wheat plus 3% whey protein concentrate were phenylalanine plus tyrosine. In further studies Forsum (320) evaluated the same whey protein concentrate as a supplement to corn,
rice and potatoes. The whey protein concentrate was a more efficient supplement for corn and rice proteins than dried skim milk; supplement efficiency was the same for potatoes.

Forsum (321) also evaluated the nutritional quality of whey protein concentrates produced by ultrafiltration and gel filtration and fractionated further by large scale gel filtration. Animal feeding studies with rats showed the α-lactalbumin rich fraction to have the highest protein efficiency ratio and net protein utilization. β-Lactoglobulin enriched fractions however had only moderate PER values although the net protein utilization was high. The author proposed that α-lactalbumin fraction be used as an ingredient in a humanized infant formula because this protein represents the main whey protein in human milk (322, 323).

Forsum (324) also showed that when the nonfat dry milk, comprising 5% of the corn soy milk formulation was replaced by whey protein concentrate, the net protein utilization was increased by 17%; the protein efficiency ratio was not given. This result had already been predicted by Vaughan (317) on the basis of the amino acid patterns.

Protein fortification with whey proteins depends on the ability of the amino acids of the whey proteins to complement the amino acids of the protein food to be fortified. As the above cited results show, the nutritional quality of all of the cereal grains studied and even of a high protein food containing soy proteins is improved to some extent by the addition of whey proteins.
a. Antinutritional factors

Whey and its fractions cannot be used as nutritive substitutes for nonfat dry milk or as ingredients in engineered foods without some consideration of the occurrence of possible adverse effects.

1. Lactose intolerance

A major problem in the utilization of cheese whey has been the high concentration of lactose. For many years, whey was disposed of by direct feeding to farm animals. However, it quickly became apparent that if too high levels were fed, severe diarrhea ensued, particularly with young animals (325, 326, 327, 328).

In humans, the consumption of lactose or of foods containing it, often leads to abdominal bloating, rumbling, cramping and diarrhea. Symptoms may occur within 30 minutes of ingestion or be delayed for up to 12 hours (329). This effect is attributed to a deficiency of lactase (β-galactosidase) in the upper gastrointestinal tract (330).

Studies in recent years have demonstrated a pattern of milk intolerance in non-Caucasian children and adults that has been attributed to low levels of intestinal lactase (329, 331). Approximately 70% of the world's adult population is lactose-intolerant (332). In the United States, about 40% of the black population show some symptoms of lactose intolerance by the time they are 10 (333). In some developing countries the pattern is much higher (334).

With the development of commercial sources of lactase enzyme, technology for the enzymatic conversion of lactose in
milk and whey is now available to the dairy industry (335). Therefore, lactose-modified milk and whey may be readily produced for incorporation into food products for the lactose-intolerant if the need arises (336).

2. Kininogen

Milk contains a protein, which, when treated with trypsin or snake venom, releases a peptide with the kinin-like ability to contract smooth muscle such as Guinea pig ileum (337). The kininogen is present in whey and has been isolated from acid whey by treatment with trichloroacetic acid to remove \( \beta \)-lactoglobulin and by salt fractionation with ammonium sulfate to remove \( \alpha \)-lactalbumin. Further purification was effected by fractionation on polyacrylamide gel (338). The presence of this kininogen could have undesirable implications for the use of whey in weaning foods for very young children; kinins are pain producing peptides (339) and the ability to contract smooth muscle could be related to the tendency of babies to "spit up" after feeding.

3. Allergy to milk proteins

There is an abundance of literature on the subject of milk allergy dating from Hippocrates to numerous recent reports. Figures in the literature for incidence of milk allergy vary widely, but the occurrence is usually estimated at less than 1% (340).

One of the more important types of allergic reactions which is manifest in human milk allergy is characterized by the presence of cell-fixing reagins (antibodies) and the liberation of
mediators of the allergic reaction (histamine, etc) by the allergen-
antibody reaction. However, few investigators have studied this;
instead the antigenicity of the milk proteins has received major
attention. It is not clear whether the same sites on the proteins
are responsible for both antigenicity and allergenicity (340).

Many early studies on allergenicity of whey pro-
teins have been criticized on the grounds that the proteins were not
sufficiently purified. For this reason, reports on specific aller-
genicity must be viewed with caution. Bleumink and Berrens (341)
showed that allergenicity of $\beta$-lactoglobulin increased after modifi-
cation by the Maillard reaction. Bleumink (342) claimed that $\beta$-lacto-
oglobulin is a major allergen in cows' milk. The author also claimed
that the increase in allergenicity because of the Maillard reaction
was due to the formation of N-glycosidic sugar-protein bonds.

The extensive study by Goldman et al (343) showed
the frequency of reactions to casein, $\beta$-lactoglobulin, $\alpha$-lactalbumin
and serum albumin by milk allergic infants and children: 60% reacted
to casein, 62% to $\beta$-lactoglobulin, 53% to $\alpha$-lactalbumin and 52% to
serum albumin. However, Goldman et al used a casein contaminated with
whey proteins so the figure for casein may be high.

Much work has been done to remove the allergeni-
city of milk proteins, usually by heat denaturation. The most common
allergens of milk were the most heat resistant milk proteins also
found to be present in antigenic form in milk formulas studied. These
were casein, $\beta$-lactoglobulin and $\alpha$-lactalbumin. Goldman et al (343)
reported that 80% of their patients had been on heat-processed milk
before and at onset of allergic symptoms. Because the whey proteins are more sensitive to heat treatment, it has been claimed that patients allergic to these proteins should only be given heat processed milk (344).

Hanson and Johansson (340) were not aware of any reports of allergic reactions due only to denatured or degraded milk proteins. However, Spies et al (345) showed that after pepsin hydrolysis, new antigenic specificities appeared in dialysates of β-lactoglobulin, α-lactalbumin, albumin and casein.

Milk proteins are absorbed from the gut in an antigenic form so formation of antibodies can occur (340). However, the presence of antibodies to milk proteins demonstrated by most of the usual tests do not correlate well to the clinical symptoms probably because the antibodies are not etiologically related to the symptoms (340). Skin tests, however, do correlate well with oral challenge (346).

It is apparent from the above results that milk allergy must also be considered when incorporating whey and its fractions into foods. Special care must be taken with infant formulas since most cases of milk allergy appear during the first few months of life and the hypersensitivity also vanishes early in life.
SCOPE OF INVESTIGATION

The objectives of this study were to:

A. Examine rehydration and suspension characteristics of commercially prepared whey soy drink mix when reconstituted for beverage use with tests routinely applied to dry milks.

B. Explore the influence of three processing parameters, homogenization pressure, total solids of the mixture homogenized and use of emulsifier in the formulation on physical properties related to rehydration of WSDM after spray drying.

C. Use response surface methodology to correlate the three process variables with selected properties including average particle size (de) after reconstitution, nitrogen solubility index, rate-of-dispersibility of the powder, phase separation on standing after reconstitution and free fat content of the powder.

D. Study factors influencing solubility of the soybean flour proteins in cheese whey.

E. Investigate means of improving soybean flour protein solubility with chemical additives.
EXPERIMENTAL PROCEDURE

A. Materials

Commercially prepared samples of whey soy drink mix (WSDM), manufactured under specifications (159) were obtained from the Sanna Division of Beatrice Foods, Menominee, Wisconsin; Foremost Foods Co., Dublin, California; Land O'Lakes, Minneapolis, Minnesota; and Maple Island, Inc., Minneapolis, Minnesota. Two samples (Foremost and Land O'Lakes) were prepared with full fat soy flour only; the other two samples were prepared with defatted soy flour. All samples contained added vitamins and minerals.

For samples prepared in the Dairy Laboratory pilot plant, soybean flour used was toasted defatted Nutrisoy purchased from Archer-Daniels-Midland Co., Decatur, Illinois. The flour contained 52% protein, 1% fat and had a PDI of 20% or less. Sweet cheese whey was obtained from the manufacture of cheddar cheese in the Dairy Laboratory pilot plant. The whey was drawn from the cheese vat into a jacketed Crepaco 330 gallon holding tank, clarified and separated with a DeLaval Model 370 Triprocess Air Tight centrifugal clarifier and separator, and then pasteurized with agitation at 145°F (62.8°C) for 30 min or at 163°F (72.8°C) for 15 sec in a DeLaval triple tube tubular heater. After cooling, the whey was held overnight at 40°F (4.4°C). If necessary, part of the whey was condensed to 16% total.
solids under vacuum and held in the cold until needed. Some whey was
condensed to 45% total solids and stored frozen. The soybean oil was
Crisco brand, purchased at a local supermarket. 42DE corn syrup
solids were Frodex brand, purchased from American Maize Co., Hammond,
Indiana. The emulsifier was polyoxethylene sorbitan monostearate
(Tween 60) with an HLB number of 14.9 and was supplied by ICI United
States, Inc., Wilmington, Delaware.
B. Sample Preparation

WSDM specifications (159) require that WSDM, manufactured using
only defatted soybean flour, shall include ingredients in the follow­
ing proportions: sweet cheese whey solids, 41.7%; defatted soy flour,
30.0%; 42DE corn syrup solids, 9.2%; and soybean oil, 19.2%.

The sweet whey may either be that produced in a normal cheese­
making operation or it may be concentrated to a liquid concentrate
and diluted back to 16% total solids for WSDM manufacture.

Three processing variables, homogenization pressure, total solids
of the mixture homogenized and use of emulsifier were chosen for in­
vestigation of their influence on the rehydration characteristics of
WSDM after spray drying.

The experimental procedure followed was a 3 x 3 x 2 factorial
design with effects of homogenization pressure and total solids of
the mixture homogenized examined at 3 levels while emulsifier addition
was examined at 2 levels, 0 and 0.5%. Lower and upper variable limits
of homogenization pressures and mix total solids were selected by
considering standard industrial practice. The emulsifier level was
selected from industrial recommendations (162).
A series of 24 samples with and without 0.5% emulsifier (Tween 60, HLB 14.9) added on a total solids basis was prepared using the de-fatted flour formulation. Ingredients were dispersed into fluid sweet whey containing 6.6% total solids or into condensed whey containing 15.9% total solids so that total solids of the liquid mixtures were 13.7% and 26% respectively. If Tween 60 was added, it was melted and dispersed into the warm whey (110°F (43.3°C)) prior to addition of the other ingredients. The mixtures were divided into thirds and pasteurized at 175°F (79.4°C) for 25 sec, subjected either to single stage or to double stage homogenization [second stage 35.2 kg/cm² (500 psi) in all cases] at 126.6 (1800), 175.8 (2500) or 211.0 kg/cm² (3000 psi), vacuum evaporated to 40% total solids and spray dried. A flow diagram of the process is shown in Figure 4.

The four batches were mixed separately in a 50 gallon (189.25 l) stainless steel jacketed holding tank equipped with an agitator. Batch sizes were 250 pounds (113.6 kg) (34 pounds (15.4 kg) dry solids) and 113 pounds (51.4 kg) for the mixtures containing 13.7% total solids and 26% total solids respectively. A Manton-Gaulin Model 4030-2 Triplex homogenizer was used for all homogenizations at 110°F (43.3°C). Pasteurization was done with a DeLeval triple tube tubular heater. Samples were condensed at 115°C (46.1°C) under vacuum in a custom made APV all stainless steel recirculating batch vacuum evaporator with an external steam heated tubular heater and a 25 gallon (94.6 l) body (evaporating capacity 15-18 gal/hr (56.8-68.1 l/hr) at approximately 90°F (32.2°C) product boiling temperature).
Experimental procedure for whey soy drink mix preparation.

Figure 4
Spray drying was done with a Bowen Engineering, Inc., table model laboratory spray dryer equipped with a water cooled spinning disc atomizer. The drying chamber was 30" x 30" (.75 x .75 meter) with a sweeper blade to move the dried powder from the flat bottom of the chamber to the collector. Concentrate was fed to the dryer with a Zenith size 5 metering pump at a rate of 76 ml/min (11 lb/hr). The inlet air temperature varied from 420 to 435°F (215.6 to 223.9°C) and the outlet temperature averaged 195°F (90.6°C). 2-3 pounds (0.9-1.4 kg) of powder were collected for each sample dried.

The pasteurizer, homogenizer and evaporator were all rinsed with hot water between samples. The spray dryer was not rinsed but was dusted and blown out with hot air between samples.

Because of operating conditions in the pilot plant, it was impossible to prepare all the samples at the same time or to prepare them in random order. All 12 samples given single stage homogenization were prepared from the same lot of whey and flour. At a later date, the 12 samples given double stage homogenization were prepared from a new lot of whey and the same lot of flour which had been stored at 40°F (4.4°C). Although the bulk of the plant operations were completed within one 5 day work week, 2 or 3 of the concentrates to be dried had to be held at 36°F (2.2°C) over the weekend before drying. Because of time considerations, concentrates were not standardized to 40% total solids before drying. No replicates were run.

A third series of 6 samples was prepared with and without the addition of 0.5% Tween 60 to test the effects of homogenization at 40% total solids on redispersibility characteristics. A flow diagram
of the procedure followed is shown in Figure 5. This processing sequence is not in compliance with the WSDM specifications.

After blending the other ingredients into the whey, it was necessary to give the mixture a preliminary single stage homogenization at as low a pressure as possible to disperse the oil before processing further. To determine what this pressure should be, small batches of the mixture were run through the homogenizer at pressures of 500 (35.2), 1000 (70.3), 1200 (84.4) and 1500 (105.5) psi (kg/cm²). The homogenized samples were then checked for oiling off by the oiling off test for cream (347).

This test was performed as follows:

1 ml of sample was pipetted into a Babcock skim milk test bottle and water at 200°F (93°C) added with mixing to within 1/2 inch (1.25 cm) of the base of the neck. The sample was then centrifuged at 800 rpm for 10 sec on the Babcock centrifuge. After centrifuging, hot water was carefully added to bring the column up to the top of the neck of the bottle, and the sample was centrifuged again for 5 min. The height of the oil layer was read off the graduated neck; a reading of three or more indicated a noticeable separation of fat. As there was no fat separation at pressures of 1200 psi (84.4 kg/cm²) and higher, 1200 psi (84.4 kg/cm²) was selected for use.

Batch sizes of the two initial mixtures were 350 pounds (159.1 kg) each (50 pounds (22.7 kg) dry solids). Each batch was pasteurized, condensed and carefully standardized to 40% total solids with water before being divided into thirds for homogenization and spray drying.
Modified experimental procedure for whey soy drink mix manufacture.
(Note: not permitted by the specifications.)

Figure 5
A new lot of whey and a new lot of flour were used for this run. No replicates were run.

All powder samples were analyzed for gross composition (moisture, fat, nitrogen, ash), solubility index, free fat, dispersibility, sinkability, bulk density and particle size distribution. After reconstitution, Brookfield viscosities, pH, NSI, average equivalent spherical diameter, fat in the soluble nitrogen fraction, and phase separation after 1 and 24 hours of standing were measured.

Total solids of the mixture that was homogenized and of the concentrate that was spray dried were also measured.

Compositions of the supernatants of the reconstituted samples homogenized double stage at 40% total solids were measured after the samples had been permitted to settle by gravity.

C. Analytical Methods

1. Microscopic examination

NDM and WSDM powders and reconstituted beverages were examined both by light microscopy and by scanning electron microscopy.

a. Light microscopy

All samples were examined with a Leitz Dialux microscope equipped with a high pressure ultraviolet lamp as well as a tungsten light source. A range of excitation filters and suppression filters permitted the use of ultraviolet light to excite the specimen to permit observation in dark field illumination of secondary fluorescence from samples that were differentially stained with fluorochromes in order to detect component distribution. The microscope was also equipped with a 35 mm camera for photographs as desired. Polarizing
filters and a retardation plate to give a background color by retarding the wavelength of the light by 1/4 of a wavelength were used for polarizing microscopy with visible light.

Intact powders were examined as follows:

1) A drop of clear nail polish was dropped on the microscope slide and a small amount of powder dispersed in it by gentle mixing with a fine glass stirring rod. A cover slip was then placed over the droplet containing the powder particles and the edges were sealed with more nail polish. Nail polish seemed to cause the particle walls to collapse slightly as judged by dark field examination.

2) To break up agglomerates, powders were dispersed in a glycerin:water solution (1:1) for examination. A drop of glycerin solution was placed on the slide and the powder dispersed in it; after placement of a cover slip, the edges were sealed with nail polish as before. Although the glycerin solution did disperse WSDM agglomerates, it also caused the particles to swell and release gas bubbles, probably because of partial solubilization of fat; this solution did not disperse NDM agglomerates, however.

3) Rehydrated WSDM samples were examined by first reconstituting the powders to 15% total solids with water and then placing a drop of sample directly onto the microscope slide. After covering with a cover slip, the edges were sealed with nail polish.

It was desirable to stain some rehydrated samples. For differential staining and subsequent examination in the ultraviolet, acridine orange and phosphine dyes were selected. Acridine
orange is a basic (cationic) stain which accumulates when negative charges are available; it will fluoresce red-orange when ionized as when staining proteins above their isoelectric point, that is, when the proteins are negatively charged. Phosphine dye is a fat stain that shows no fluorescence in the absence of fat but will cause fat droplets to fluoresce bright yellowish-green ($3^\text{4}8$).

Rehydrated samples could not be satisfactorily stained by mixing stain with the sample. The most effective technique was to place a drop of sample onto the microscope slide and cover it with a cover slip. Then, a drop of a 0.1% aqueous stain solution was placed at the edge of the cover slip; the stain was drawn under the cover slip by capillary action. Excess stain was gently blotted away and the cover slip edges were sealed with nail polish.

To visualize both fat and protein at the same time, the samples were first stained with acridine orange and then with phosphine; the background color was overwhelming if the phosphine was added first or if the dyes were mixed and then added.

Best observations in the ultraviolet were made through the deep blue filter with a K 510 barrier filter also in place.

4) To examine powder particles as they just began to dissolve, an agar mounting technique was employed. Water solutions containing 1% agar were brought just to the melting point and the samples were quickly dispersed in them. A drop of the warmed solution was then placed on a cooled microscope slide where it solidified. If it was desired to stain the sample, the stain was dispersed in the agar solution before the powder was added. 0.5% phosphine in agar
gave the most satisfactory results.

The samples had to be examined immediately after preparation because gradual dissolution of the powder particles occurred on standing. Nonfat dry milk particles could not be examined by this technique because the powder wetted and dissolved completely during slide preparation.

b. Scanning electron microscopy (SEM)

For examination of intact powders, specimen holders were covered with double-faced cellophane adhesive tape and the powder particles sprinkled onto the tape where they stuck. After removal of excess powder, the samples were coated with carbon followed by gold and then examined in a JEOL JSM-50A scanning electron microscope equipped with a Polaroid camera.

Reconstituted samples were examined by reconstituting to 15% total solids. Thin films of well mixed samples were spread directly onto the specimen holders and air dried before coating with carbon and gold.

2. Particle size distribution

a. Optical microscopy

The particle size distribution was measured on all powders by optical microscopy with a Bausch and Lomb microscope equipped with a Vickers A.E.I. Image Splitting Measuring Eyepiece.

In the Image Splitter, a prism system is interposed between the microscope objective and the eyepiece to produce a double image of the field of view. This prism system is precisely rotatable by a micrometer screw. On rotation of the prisms, double images of
the objects to be measured traverse one another. Measurement is accomplished by reading off the micrometer the amount of prism rotation necessary to place the double image of the particle exactly edge to edge in the axis of desired measurement. Measurement is accomplished in the plane of the object and is completely free of errors due to parallax, differences in contrast, etc. Color filters are included so one of the double images is green and the other red; this makes measurement very easy, especially in a crowded field.

For measurements, the powder particles were randomly dispersed in nail polish on a microscope slide and covered with a cover slip; dispersal was such that 50 particles or less were visible in each field viewed. All measurements were carried out with an objective power of 43 x (N.A. 0.65); this gave a reading accuracy with the micrometer scale on the image splitter of 0.325 micrometer. The micrometer was calibrated with the desired objective lens with a stage micrometer before powder particle measurements were made.

The method of measurement used involved setting the image splitter to a zero shear position, the point where no double images can be seen. After noting the initial reading on the micrometer drum, object images were traversed across each other until they were set edge to edge. After noting the final reading, the difference in readings was multiplied by the calibration factor to yield the diameter of the object measured in micrometers.

Particle diameters were measured using Martin's diameters, the mean length of a line parallel to the direction of scan that divided the particle profile into two equal areas (349).
After measurement of the diameters of about 400 to 600 particles for each sample, the particles were tallied into different size classes each of which was divided into 2 micrometer intervals. The diameter $d$ representing the size class was taken as the arithmetic mean of the class limits (e.g. 0-2, $d=1$; 2-4, $d=3$). A statistical analysis was then made in terms of the number of particles of different sizes and plotted in a number of ways, starting with a histogram (size-frequency or size-distribution curve). The number, $n$, of particles within each class was plotted against $d$. The narrower the class intervals the more the histogram approached a curve.

Cumulative percentage distribution curves were also plotted. This included the percentage of the total number of particles, $100 \times \frac{\sum_{d} n}{n}$, plotted against $d$; also included were the percentage of the total surface, $100 \times \frac{\sum_{d} nd^2}{\sum_{d} n}$, and the percentage of the total volume, $100 \times \frac{\sum_{d} nd^3}{\sum_{d} n}$. This type of curve can conceal the bimodal character of a powder (350).

A Fortran computer program was drawn up for calculations with ERRC's IBM 1130 computer. Calculations included the numerical average diameter or arithmetical mean $d_1 = \frac{\sum_{d} nd}{\sum_{d} n}$; this was only of statistical interest because $d_1$ is too strongly influenced (decreased) by the smaller class sizes. Linear dimensions were given by $d_2 = \frac{\sum_{d} nd^2}{\sum_{d} n}$. The average particle size with respect to surface for a sample was given by $d_3 = \frac{\sum_{d} nd^3}{\sum_{d} n^2}$; it is this value that should be used in calculations of specific surface of a powder. The average particle size with respect to volume was calculated as $d_4 = \frac{\sum_{d} nd^4}{\sum_{d} n^3}$. 
The average volume per particle, determined from the total number of particles per unit volume, is used to give the average particle diameter \( \bar{D} = \sqrt[3]{V_{\text{avg}}/n}. \) This value is the same as the average equivalent spherical diameter, \( d_e \), measured directly by the Coulter counter. The average surface per particle was used similarly to give the average particle diameter \( \bar{A} = \sqrt{A_{\text{avg}}/n}. \)

Cumulative plots were also made with log probability paper to determine if the particle size distributions were log normal.

An illustration of a complete calculation and graphical plots for one sample may be found in Appendix A.

b. Coulter counter measurements

Particle size distributions were measured on samples reconstituted with water to 15% total solids with a Coulter counter model A equipped with a 280 micrometer aperture. The electrolyte used for all measurements was 0.25% sodium chloride preserved with 0.25% formaldehyde. Because of the size of the aperture and electronic noise in the instrument it was not possible to measure particles smaller than 6-8 micrometers in diameter.

The Coulter counter method determines a number-volume particle size distribution of particles suspended in an electrically conductive liquid. The suspension passes through a small aperture having an immersed electrode on either side. The particle concentration is low enough so that the particles will pass through the aperture one at a time.

Each particle passage displaces electrolyte within the aperture, changing the resistance between the electrodes; this
produces a voltage pulse, proportional to particle volume. The series of pulses is amplified, scaled and counted automatically, using pulse height analysis (196).

The average equivalent spherical diameter, \( \overline{d} \), \( \left( \frac{\sqrt[3]{3\text{vol}}}{4\pi n} \right) \) was calculated and cumulative frequency plotted by computer as percentage above the stated size vs log d. \( \overline{d}_1, \overline{d}_2, \overline{d}_3, \overline{d}_4 \) and \( \overline{A} \) were computed from the data; log probability plots were also prepared.

An illustrative data sheet and complete calculation for one sample are shown in Appendix B.

3. Total solids

Total solids were determined on all fluid samples by the standard Mojonnier procedure (347).

One to two grams of well mixed sample was weighed into an aluminum solids dish. The sample was spread in a thin film on the bottom of the dish and water uniformly evaporated from the sample by placing the dish on the Mojonnier tester hot plate at 180°C. The dish was transferred into the solids vacuum oven at 100°C and heated for 10 min under 25 inches of vacuum.

After cooling in the cooling desiccator for 5 min, the dish was weighed. Total solids percentage was calculated as weight of solids divided by fluid sample weight times 100.

All analyses were run in duplicate.

4. Total moisture

The moisture content was measured on all powders as follows:

One to 1.5 g of powder was weighed into a dried weighed 70 mm glass weighing bottle equipped with a ground glass top. After
weighing, the cover was loosened and the bottle containing the sample was placed onto the metal shelf in a vacuum oven at 65°C and dried overnight under pressure ≤ 100 mm of mercury. The vacuum pump was stopped and dried air was admitted into the oven. The glass top was placed tightly on the weighing bottle and the bottle was then removed to a desiccator and cooled for 1 hour before weighing. The percentage of moisture was calculated as the weight of the water lost divided by the weight of the sample before drying times 100. All samples were analyzed in duplicate.

5. Total fat

Total fat content of all samples was determined by the Mojonnier modification of the Rose-Gottlieb procedure for fat extraction (347).

If the sample to be analyzed was a powder, one gram was weighed into a Mojonnier extraction flask and 9 ml of hot distilled water added, followed by vigorous shaking to dissolve the sample. If the sample was fluid, 10 g was pipetted directly into the flask with a 10 ml pipette; if the sample was a concentrate, 2 to 5 g were pipetted into the flask followed by sufficient hot distilled water to make 10 ml.

Next, 1.5 ml of concentrated ammonium hydroxide was added to the sample. After thorough mixing, 10 ml of 95% ethanol was added; after corking, the mixture was shaken for 1.5 min. 25 ml ethyl ether was added and the mixture was shaken for 1.5 min. 25 ml petroleum ether (b.p. 49-60°C) was added and the mixture was shaken for a further 1.5 min.
After centrifuging for 30 sec, the ether mixture containing the extracted fat was decanted into a weighed Mojonnier fat pan; the solvent was evaporated off on an electric hot plate at 135°C.

For the second extraction, 5 ml ethanol were added followed by 15 ml each of ethyl and petroleum ethers; the mixture was shaken for 30 sec after addition of each reagent and centrifuged as before. The solvents were decanted into the same fat dishes as were used for the first extraction and were evaporated on the hot plate as before. A third extraction was not done.

The last traces of solvent were removed in the Mojonnier tester fat oven at 135°C for 5 min under 25 inches of vacuum. The pans were then cooled in the cooling desiccator for 7 min and weighed.

Percentage fat was calculated by dividing the weight of fat by the weight of sample times 100.

All samples were run in duplicate.

6. Free Fat

Free fat was measured in all powders as the percentage of the total fat extracted by carbon tetrachloride¹ according to the method of Tamsma et al (228) for foam-dried whole milk.

At room temperature, 50 ml of carbon tetrachloride were pipetted onto 10 g WSDM in a 125-ml glass-stoppered Erlenmeyer flask. The mixture was then shaken for 30 min on a Cherry-Burrell wrist-action shaker at an intensity setting of 5. Immediately upon cessation of

¹Carbon tetrachloride is readily absorbed through the skin and lungs and can cause severe liver damage if carelessly handled.
shaking, the samples were filtered through fluted 18.5 cm Reeve Angel No. 802 filter paper. A 25-ml aliquot was then transferred into a weighed Mojonnier fat pan and the solvent evaporated off on a 135°C hot plate in the hood. The last traces of solvent were removed in a 100°C vacuum oven for 7 min according to the standard Mojonnier procedure for fat measurements. After cooling, the fat residue was weighed and the weight multiplied by two.

Duplicate analyses were run.

7. Total nitrogen

Total nitrogen was determined on all samples by a micro Kjeldahl procedure (23). 30 mg samples were weighed into micro Kjeldahl flasks for analysis if the samples were powdered. Total protein was calculated by multiplying the per cent total nitrogen by the factor 6.25. All samples were analyzed in duplicate.

8. Nitrogen solubility index (NSI)

Protein dispersibility was determined on all powder samples by the official procedure for the determination of nitrogen solubility index (NSI) (351).

Five grams of dry sample were weighed into a 400 ml beaker. Two hundred ml of water at 30°C was added, a little at a time, and the sample was thoroughly dispersed with a stirring rod; the last of the water was used to wash off the stirring rod.

The mixture was then placed in a thermostatted water bath at 30°C and stirred at 120 rpm for two hours with a stainless steel paddle that was a segment of a circle, with an extreme diameter of 50 mm. The mixture was then quantitatively transferred to a 250 ml
volumetric flask, diluted to the mark with water and mixed thoroughly.

After cooling to room temperature, the dilution was checked and adjusted if necessary. The sample was mixed thoroughly, poured into 50 ml glass centrifuge tubes and centrifuged in an International centrifuge for 10 min at 1500 rpm. The supernatant was decanted and collected.

Five ml aliquots of the supernatants were pipetted into micro Kjeldahl flask; 2 ml aliquots of the well mixed original samples were taken. Total nitrogen and total water soluble nitrogen were then determined by the standard micro Kjeldahl procedure (23).

The nitrogen solubility index (NSI) was computed as the water soluble nitrogen divided by the total nitrogen times 100.

NSI was determined in fluid samples by the following procedure:

Sufficient well mixed fluid sample to yield about 5 g of total solids was weighed into a 250 ml volumetric flask and diluted to volume with water. After mixing and one hour's standing at room temperature, the sample was centrifuged and analyzed as before.

Duplicate analyses were run in all cases.

9. Total ash

Ash was determined by weighing 1 to 2 g powder into porcelain evaporating dishes followed by ignition in a muffle furnace at 550°C overnight. After cooling, the dishes were weighed and percentage ash was calculated by dividing the weight ash times 100 by the sample weight. All samples were run in duplicate (23).
10. pH

pH was measured at room temperature on all samples with a Beckman Research pH meter with glass and calomel electrodes. Powders were reconstituted to 15% total solids for pH measurement.

11. Viscosity

All viscosities were measured with a Brookfield Synchro-electric Model LVT viscometer equipped with a UL adapter plus cup for low viscosity range from 0-100 centipoise (CP). Although the use of a Brookfield viscometer presumes Newtonian behavior of the fluid, in the low viscosity range, deviations from Newtonian behavior are generally considered to be minimal. The purpose of this study was to examine relative differences in viscosity induced by processing rather than absolute viscosity values.

WSDM powders were prepared for viscosity measurements by reconstitution with water at room temperature to 15% total solids. After standing at room temperature (20-27°C) for one to two hours, the samples were refrigerated overnight. Samples were then removed from the refrigerator and equilibrated to room temperature before viscosity was measured. Because of the tendency for phase separation on standing, the samples were mixed thoroughly before measurements were made. Since the viscosities of all reconstituted powders were below 100 CP, the UL adapter and cup were used. Viscosities were measured at 6, 12, 30 and 60 rpm. At 6 and 12 rpm, measurements were made at 30 second intervals; at the higher speeds, measurements were made after 3 revolutions of the dial. At least two measurements were made at each speed.
The viscosities of concentrates and other fluid samples prepared in the pilot plant were measured on the day of preparation after equilibration to room temperature. For viscosities below 100 CP, the UL adapter was used; for viscosities between 100 and 1000 CP, it was necessary to use the #2 spindle and the #3 spindle for viscosities greater than 1000 CP. Viscosities were measured at the 4 different speeds as described above.

In some cases, samples of concentrates with total solids greater than 15% were diluted to 15% total solids. After equilibration for one hour at room temperature, viscosity was measured with the UL adapter as before.

12. Sinkability

Sinkability was used as a measure of the wettability of the powders and was determined by the procedure of Bullock and Winder (259).

To carry out the test, 500 mg of powder were weighed into a glass bottle 2 cm wide and 5 cm deep, equipped with a screw cap with a 1.5 cm hole covered with a piece of stainless steel 20 mesh screening. 50 ml of distilled water at 25°C were placed in a funnel equipped with a pinch clamp on a tube on the stem. A 125 ml Erlenmeyer flask was positioned below the outlet. The powder was sprinkled quickly and evenly onto the quiescent surface of the water through the screen; timing began when the powder first struck the water surface. After 30 sec, the pinch clamp was opened and that portion of the powder that had sunk through the water surface was quickly drawn off into the flask, leaving behind the material that was still floating.
Approximately 40 ml of water were removed, usually within 30 sec.
After complete dispersal of the sample in the flask, a 10 ml aliquot was pipetted into a weighed Mojonnier total solids pan and total solids were measured by the Mojonnier procedure (347). The weight of total solids found multiplied by 1000 yielded the per cent sinkability of the sample.

All samples were analyzed in duplicate.

13. Dispersibility

Dispersibility was measured on all powders with a modification (267) of the procedure of Sinnamon et al (211) who modified the procedure of Stone et al (221).

In the original method of Stone et al, 15 g of milk powder were added to 90 ml of water at 70-75°F (21.1-23.9°C) in a 250 ml beaker. The mixture was stirred by hand with a teaspoon for a designated time interval; after stirring, the mixture was filtered through a coarse (40-60 micrometers) fritted glass Buchner funnel with 16 inches of vacuum. The filtrate was diluted to 200 ml and grams of solids were measured in a 20 ml aliquot. When multiplied by 10, this weight represented the dispersibility for the stirring time selected.

Sinnamon et al modified the procedure to standardize the filtration step, to detect smaller differences among dried milks and to improve the precision of the procedure.

The temperature of the water was reduced to 38°F (3.3°C); this permitted the detection of smaller differences among milk samples. The filtration step was improved by using scalper filters
consisting of conical screens of 100-mesh (140 micrometers) and 150-mesh (103 micrometers) stainless steel just ahead of the vacuum filter to eliminate filter clogging; this reduced filtration time to within 15 sec. Only 35 ml of filtrate were collected for analysis and the dilution step was eliminated; by removing only the minimum filtrate needed for total solids analysis, the solution was separated from the undispersed material more quickly.

Although the above procedure is not the official method for the determination of the dispersibility of nonfat dry milk (352), it has been used routinely in the Dairy Products Laboratory for many years.

The modification of Kontson et al simply consisted of stirring the sample in a 300 ml stoppered baby bottle by rotation at 120 rpm for 30 sec instead of stirring by hand; the precision of the method was increased further by this modification.

To determine the rate-of-dispersibility of WSDM samples, the following procedure was followed:

Seven g of WSDM was added to 93 g of distilled water at 4°C in a 300 ml wide mouth baby bottle. The bottle was quickly capped and the sample stirred by rotation for 30 sec at 120 rpm. The entire sample was immediately filtered through two conical screens of 100-mesh and 150-mesh into a 125 ml Erlenmeyer flask. Total solids were then measured in the filtrate by the Mojonnier procedure (347).

To calculate the percentage dispersed, the following equation was used:
\[
\text{% dispersibility} = \frac{\frac{100 \times 93 \text{ g water}}{\text{100-Total solids of the filtrate}} - 93 \text{ g water}}{\frac{100 - \text{% moisture in sample}}{100} \text{ sample wt in g}}
\]

It was necessary to reduce the concentration of WSDM dispersed from 15 to 7 g in order to maintain the rapid filtration rate. Because the insoluble constituents of soy flour, even though dispersed, immediately clogged the Buchner funnel, this filtration was eliminated; therefore all the filtrate that passed through the wire screens was collected for total solids analysis.

14. Solubility index

Solubility index was measured on all powders with a modification of the official procedure for dry whole milk (274). Seven g WSDM was weighed and added to 93 ml distilled water at 75°F (23.9°C) in the special mixing jar as specified by the American Dry Milk Institute. The jar was placed in the mixer (special type, as specified by the American Dry Milk Institute) and the solution was stirred for exactly 90 sec. The mixed sample was transferred to a 250 ml beaker and permitted to stand 5-15 min until the foam had separated sufficiently for its complete removal with a teaspoon.

After foam removal, the sample was mixed thoroughly for five seconds and poured into a conical glass centrifuge tube graduated in 0.1 ml divisions from 0 to 1.0 ml, 0.2 ml divisions from 1.0 to 2.0
ml, in 0.5 ml divisions from 2.0 to 10.0 ml, in 1.0 ml divisions from 10.0 to 20.0 ml and a 50 ml mark 1/2 inch (1.25 cm) from the top of the tube.

The sample was then centrifuged for 5 min at 800 rpm in an International Centrifuge equipped with a head 18 inches (45 cm) in diameter. After centrifugation, the supernatant was decanted and discarded, 25 ml distilled water at 75°F (23.9°C) was added and the sediment layer dislodged by shaking. The tube was then filled to the 50 ml mark with water, the contents mixed and the sample recentrifuged at 800 rpm for 5 min.

After centrifuging, the tube was held in a vertical position and solubility index reported as milliliters of sediment in the tube to the nearest graduated scale division.

Samples were run in duplicate.

15. Bulk density

For the measurement of bulk density, a 100 ml plastic cylinder, graduated in 1.0 ml divisions was cut off at the 100 ml mark and weighed. A powder funnel was placed on a ring stand and positioned 1/2 inch (1.25 cm) above the weighed cylinder. The test sample was transferred into the funnel and allowed to slide into the cylinder by gravity. When the cylinder was full, the top was levelled with a spatula and the net weight determined. Bulk density was then reported as g per ml (353). Duplicates were run in all cases.

16. Settling (suspension stability)

Measurement of the settling after reconstitution, that is, phase separation, is required by the WSDM specifications (159).
Settling characteristics were measured as follows:

A 15 g quantity of WSDM was added to 85 ml of distilled water at 75°F (23.9°C). Reconstitution was carried out by stirring with a teaspoon until all lumps were completely dispersed. The reconstituted sample was allowed to stand undisturbed for 30±5 min after which it was gently stirred with a teaspoon for 5 sec. A 25 ml aliquot was then transferred into a 25 ml graduated cylinder graduated in 0.5 ml divisions. Settling was determined as the volume of supernatant liquid accumulated after 60 min undisturbed holding. If settling is greater than 5.0 ml, the contractor takes a price loss.

For the measurement of settling after 24 hours in the cold, samples were reconstituted as described above and permitted to stand for one hour at room temperature before being refrigerated in 250 ml beakers. Use of the beakers eliminated the problem of wall effects which become important during settling in cylinders less than 25 mm in diameter (354). After standing undisturbed for 24 hours in the cold, samples were removed from the refrigerator and the total height of the liquid and the height of the supernatant liquid were measured in millimeters with a ruler divided in 0.5 mm divisions. The height of supernatant liquid was expressed as a percentage of the total height, the larger numbers reflecting greater settling.

Settling of all samples was measured in duplicate.

17. Electrophoresis

a. Polyacrylamide disc gel electrophoresis was run on the supernatants of the commercial WSDM samples with a Canalco apparatus, according to manufacturer's instructions, using a standard separating
gel at pH 9.5, by Merton Groves of the Dairy Laboratory, USDA. Samples for analysis were prepared by reconstituting the powders to 15% total solids and permitting them to settle by gravity while standing undisturbed for one hour at room temperature. The supernatants were collected by aspiration and samples taken for compositional analysis. The remaining supernatants were exhaustively dialyzed in the cold against 8 changes of distilled water for 48 hours and freeze dried. A sample of toasted defatted soybean flour was dispersed at 5.5% total solids in water and prepared for analysis in a similar manner. Fresh fluid sweet whey was dialyzed and freeze dried for use as a control.

b. Gel electrophoresis in a vertical starch bed was run on the dialyzed freeze dried samples described above by P. M. T. Hansen, Department of Food Science and Nutrition, The Ohio State University. Samples were evaluated according to the standard procedure (355) except that the starch bed was cast with 12% starch instead of 11.4% and the samples were stained with nigrosine instead of Amidoschwartz after completion of the electrophoresis.

In addition, for analysis by this technique, toasted defatted soybean flour was dispersed in fresh fluid sweet whey at total solids level of 5.5%, permitted to settle and prepared for analysis as before.

The same flour was dispersed in water at 5.5% total solids and divided into thirds. One part served as the control, the second part was treated with 2-mercaptoethanol (10 mM/l) and the third part homogenized double stage at 110°F (43.3°C) and 3000-500 psi (211.0-35.2 kg/cm²). These samples were then centrifuged at 1500 rpm for
10 min in an International centrifuge and the supernatants collected and prepared for electrophoretic analysis as previously described.

18. Gas chromatography

Lipids were extracted from the WSDM powders either with carbon tetrachloride (free fat) or by the Mojonnier procedure (total fat); samples were taken to dryness under a stream of nitrogen gas. Preparation of the methyl esters of the extracted lipids and subsequent gas chromatographic analysis were carried out by Mr. Francis Luddy of the Animal Fats Laboratory, ERRC, according to his own procedure (356).

D. Statistical Analysis

1. Analysis of variance

The data from 30 powder samples were examined for statistical significance by analysis of variance using the statistical package supplied with a Tektronix 40 51 computer. Data from the 12 samples processed with single stage homogenization were considered separately from data for samples processed by double stage homogenization.

2. Linear Regression

Simple linear correlations of y on x were determined for some data by a least squares method (357). Only data from samples processed by double stage homogenization were examined. Data pairs tested for linear correlation were: a) phase separation (1 hr) and average equivalent spherical diameter (de) of the reconstituted powder; b) phase separation (2 hr) and de; c) dispersibility and de; d) dispersibility and average particle diameter $\bar{d}$ with respect to
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surface area of the dry powder \( \left\{ \sqrt{2 \pi \text{nm}^2} \right\} \); e) average particle diameter

\( \bar{D} \) with respect to volume of the dry powder \( \left\{ \sqrt{\frac{\text{nm}^3}{\text{cm}^3}} \right\} \); f) free fat and moisture content; g) sinkability and moisture content; h) phase separation (1 hr) and viscosity (30 rpm); and i) phase separation (24 hr) and viscosity (30 rpm).

3. Response surface analysis (RSM)

After completion of the analysis of variance and the linear regressions, the five dependent variables: dispersibility, free fat content, de, NSI and phase separation, after 24 hours of standing after reconstitution were chosen for further analysis by response surface methodology (RSM). Only data obtained for those powders homogenized double stage during processing were used.

RSM is based on the assumption that when \( n \) factors (independent variables) are being studied in an experiment, the response (dependent variable) is a function of the levels at which the \( n \) factors are combined or \( y = (x_1, x_2, ..., x_n) \) where \( y \) is the response function (358 cited in 359).

Care must be used in the selection of the number of independent variables because if there are more than three, the geometrical representation may be only partly used. However, canonical transformations of the surface equations will simplify them for interpretation. The polynomial used depends on the accuracy needed and on the contribution of extra terms necessary for proper fitting measured, for example, by analysis of variance.
For three independent variables, the data may be fitted with a quadratic polynomial of the type

\[ y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3. \]

\[ \ldots \ldots 1 \]

\( b_0 \) is the center point; \( b_1 x_1 + b_2 x_2 + b_3 x_3 \) take linear effects into consideration, \( b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 \) consider second order effects and \( b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 \) consider interaction effects.

Surface contours are obtained by making one independent variable constant and solving the equation as a quadratic in the other two. If the value of the fixed variable is then altered, a set of contoured planes can be constructed into a three dimensional representation.

RSM has been used by several authors to describe the effects of processing parameters on complex food systems (105, 359, 360, 361, 362, 363).

Regression equations were obtained from the data pertaining to the 5 dependent variables chosen by fitting a quadratic equation of the form

\[ y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1 x_2 + b_{44} x_2 x_3 + b_{12} x_1^2 + b_8 x_2^2 + b_9 x_3^2 \]

\[ \ldots \ldots 2 \]

where

\( x_1 = \) emulsifier level
\( x_2 = \) homogenization pressure
\( x_3 = \) total solids of the mixture homogenized
and \( y \) is one of the 5 dependent variables.
Terms were dropped from the right hand side of the model until every term remaining contributed significantly to the fit of the experimental data. Equations obtained in this way represented the most economical equations in the number of terms that gave a significant relationship. However, all terms were included in the generation of the contour plots so as to consider all possible interactions.

Curves were generated at the Washington Computer Center, Beltsville, Maryland, using a statistical package developed by the SAS Institute (364).

Response surface predictions from the regression equations were tested in the pilot plant. A flow diagram of the processing sequences followed is shown in Figure 6. Replicate samples were processed for each set of conditions selected.

Set I served as the control and was processed according to the WSDM specifications. Batch size was 102 pounds/batch (46.4 kg/batch) at about 14% total solids. Rate-of-dispersibility was maximized under these conditions according to the computer prediction.

Set II was processed under conditions of homogenization pressure (double stage, 3000-500 psi (211.0-35.2 kg/cm²)) and total solids of homogenization (16-23% total solids) selected to maximize NSI of the reconstituted powder. Batch sizes were 75 pounds/batch (34.1 kg/batch).

Homogenization pressure (3000-500 psi (211.0-35.2 kg/cm²)) and total solids of homogenization (40%) for processing Set III were selected to minimize the free fat content of the powder and the phase separation on standing after reconstitution. Batch size was 54 pounds/
Experimental procedure for test of response surface predictions.

Figure 6
batch (24.5 kg/batch).

All powders were analyzed for composition and all physical properties measured as was done for the earlier samples.

Brookfield viscosities, average de, NSI and phase separation after 24 hours in the cold were measured on all concentrates. Concentrates of Sets II and III were diluted to 15% total solids with water for measurements of de and phase separation; Brookfield viscosities were measured both before and after dilution. NSI was measured as previously described.

E. Supplementary Experiments

1. Homogenization

A number of separate experiments were carried out in the pilot plant to evaluate the effects of homogenization on the WSDM system more precisely. The Manton-Gaulin Model 4030-2 Triplex homogenizer was used for all experiments.

a. Experiment 1

The power input into the homogenizer was measured by inserting an ammeter with a strip chart recorder into the power line. For a base line, the power input was measured with hot water recirculating through the homogenizer at the pressures to be used for the WSDM samples.

Small batches of concentrates for homogenization were mixed at three total solids levels as previously described except that a frozen whey concentrate containing 30.29% total solids was used as the starting material after thawing. Samples were prepared with and without 0.5% Tween 60.
After attachment of a two gallon conical stainless steel container to the homogenizer and preheating of the block with hot water, a one gallon (4 l) sample of well-mixed material at 110°F (43.3°C) was placed in the container. After recirculation to clear residual water out of the lines, the desired pressure, either single or double stage was applied. Two-liter homogenized samples were collected. The homogenizer was thoroughly flushed with hot water between samples. The samples were homogenized in random order. No replicates were run.

After cooling to room temperature, Brookfield viscosities were measured on all samples. In addition to the UL adapter, spindles No. 1, 2 and 3 were also used when necessary. Samples were then chilled to 40°F (4.4°C) and stored in the cold.

Measurements of de were made on all concentrates with the Coulter counter as previously described. High solids concentrates were diluted to 15% total solids with water before measurements were made.

b. Experiment 2

The effects of homogenization pressure on NSI of soybean flour in water and whey, both with and without 0.5% Tween 60 were evaluated in another series of experiments. Defatted soy flour was dispersed in water at 110°F (43.3°C) to yield a mixture containing approximately 5.5% total solids and held for 1 hour. This total solids level was chosen so that the protein concentration would be equivalent to the total protein concentration found in WSDM reconstituted to 15% TS. The same amount of soy flour was added to sweet whey
that had been condensed to 45.94% total solids, stored frozen, thawed and diluted to 6.1%-6.3% total solids with water and heated to 110°F (43.3°C). 0.5% Tween 60, the same amount used in WSDM preparation, was added to the warmed water or whey before soy flour addition.

Since difficulties were encountered in completing sample analyses before sample deterioration occurred because of lack of pasteurization, samples were prepared and analyzed 6 at a time; homogenizations were randomized within each set of 6. No replicates were run.

Homogenizations were carried out at 110°F (43.3°C) as described in Experiment 1.

All samples were cooled to room temperature and Brookfield viscosities were measured as previously described. NSI determinations were also made as previously described.

c. Experiment 3

The effects of the pH of the sweet whey used in product manufacture on NSI of the soybean flour after homogenization were evaluated. Tween 60 was omitted in this series of experiments.

A preliminary experiment was carried out as follows: Whey containing 45.29% total solids was diluted to 6.54% total solids with water. The pH of 200 ml aliquots was then adjusted to the desired value with 85% lactic acid so that pH ranged from 6.13 (control, no lactic acid added) to 5.13. 95.5 g of each aliquot were weighed out and 4.5 g of defatted soybean flour were added to each. Final pH after flour addition and standing overnight in the cold ranged from 6.56 (control) to 6.10. Each sample was then washed into a 250 ml
volumetric flask, diluted to volume and NSI's determined. Since NSI results varied so little [33.65% (control) to 32.39% (pH 6.10)], it was decided to ferment the whey to the desired pH naturally.

Fermentations were carried out as follows:

Sweet whey from freshly Cheddared 2% milk was drawn from the vat, clarified, and four 10 gallon cans drawn from the holding tank; this process took approximately 2.5 hours.

The first can was pasteurized immediately at 145°F (62.8°C) for 30 minutes for a control; pH after pasteurization was 6.31. Cans 2, 3 and 4 were reheated from 80°F (26.7°C) to 100°F (37.8°C) and held there for fermentation. Each can was pasteurized at 145°F (62.8°C) for 30 minutes immediately upon reaching the desired pH, chilled to 40°F (4.4°C) and stored in the cold until needed.

The rate of acid development with time is shown in Figure 7. Final pH's of each of the three fermented samples were 5.83, 5.23 and 4.69.

Titratable acidity was measured on each whey sample as follows:

A 5 ml aliquot was pipetted into a 125 ml Erlenmeyer flask and diluted with 50 ml of distilled water. 0.5 ml of a 1.0% phenolphthalein solution in 95% ethanol was added and the sample was immediately titrated with 0.02 N NaOH until a faint pink color persisted for 30 sec. Titratable acidity was reported as mg lactic acid per 100 ml whey and calculated as follows:

\[
\text{mg/100 ml lactic acid} = \left( \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{mg wt lactic acid}}{\text{ml sample}} \right) \times 100
\]
Acid development in fresh sweet whey.

Figure 7
The calcium content of the whey was measured by using a procedure to measure calcium ion in the presence of large amounts of phosphate ion (365, 366).

A 20 ml aliquot of whey was pipetted into a 250 ml beaker and mixed with 1 ml of gelatin solution prepared by dissolving 0.025 g gelatin in 25 ml water. Phosphate was precipitated by adding 30 ml of a molybdate reagent. This reagent was prepared as follows:

Solution I: 50 g molybdic oxide were dissolved in 200 ml distilled water and 47 ml concentrated ammonium hydroxide. A slight odor of ammonia should be apparent; if not, sufficient ammonium hydroxide should be added to give a slight excess. The solution was then filtered.

Solution II: 200 ml of 70% nitric acid were mixed with 300 ml water.

To prepare the molybdate reagent, 10 ml of solution I was mixed into 20 ml of solution II just before addition to the gelatin treated sample.

After standing for 10 min, the treated sample was filtered through Whatman No. 42 fluted filter paper.

A 5 ml aliquot of the filtrate was pipetted into a 125 ml Erlenmeyer flask and 10-15 ml distilled water were added. 5 ml of a 5N NaOH solution were added, followed by 2 drops of fluorescent calcein indicator. The solution was then titrated to the point where the yellow-green fluorescence just disappeared and changed to yellow-brown with an aqueous solution of disodium ethylene diamine tetraacetic acid that was standardized with a standard solution of calcium chloride so
that 1 ml equalled 0.300 mg calcium ion. Calculations were done as shown:

\[
\text{mgCa}^{++}/\text{whey} = \left( \frac{\text{ml EDTA}}{\text{ml sample}} \times \frac{\text{mgCa}^{++}/\text{ml EDTA}}{100} \right)
\]

Again, it was impossible to prepare and analyze all samples at the same time. All work was completed within 1.5 days, however. Samples were prepared and analyzed in sets of 6; homogenizations were randomized within each sample set.

For each set, 4.12 lbs (1.87 kg) of defatted soy flour were dispersed into 71 lbs (32.3 kg) of whey preheated to 90°F (32.2°C); after standing for 1 hour at 90°F (32.3°C), the samples were heated to 110°F (43.3°C) and one gallon (4 l) aliquots of well mixed sample drawn off and homogenized as described in experiment 1. Total solids of the wheys ranged from 6.53% to 6.58%; solids of the whey plus soy flour mixtures homogenized ranged from 11.24% to 11.51%. No replicates were run.

After cooling to room temperature, viscosities and NSI were measured on all samples as before.

d. Experiment 4

Alterations in the NSI of defatted soy flour by homogenization were investigated further by combining homogenization with pretreatment with the disulfide bond reducing agents 2-mercaptoethanol and sodium bisulfite. The quantity of reducing agent to be used was determined in a preliminary experiment by dispersing defatted soy flour in water (5.43% total solids), treating aliquots of the
mixtures with increasing concentrations of reducing agents and determining NSI after holding for three hours at room temperature. The results are shown graphically in Figure 8. On the basis of these results it was decided to use 7.5 mMol/l sodium bisulfite and 10 mMol/l 2-mercaptoethanol.

For sample preparation, 3.05 lbs (1.39 kg) toasted defatted soy flour were dispersed in 52.5 lbs (23.9 kg) warm water at 90°F (32.2°C). Two 8-liter aliquots of the well mixed sample were removed; the first aliquot was treated with 2-mercaptoethanol (10 m moles/l) and the second with sodium bisulfite (7.5 m moles/l). All samples were held at room temperature for 3 hours during which time they cooled from 90°F (32.2°C) to 81°F (27.2°C).

After preheating to 110°F (43.3°C), a control (untreated) and the treated samples were homogenized as described in experiment 1, except that only one homogenization pressure, double stage at 3000-500 psi (211.0-35.2 kg/cm²), was used throughout.

The same experiment was carried out with soy flour dispersed in whey. A frozen whey concentrate (45.94% total solids) was thawed and diluted to 6.56% total solids. After preheating to 90°F (32.2°C), samples were prepared as described above.

Brookfield viscosities and NSI's were measured on all samples, before and after homogenization as previously described.

Two 100 g aliquots of each of the two homogenized controls (untreated) prepared above were weighed into 250 ml beakers. One set was treated with 2-mercaptoethanol (10 m moles/l) and the second with sodium bisulfite (7.5 m moles/l). After holding at room
Effect of additive concentration on NSI of soybean flour in water.

Figure 8
temperature for three hours, the samples were refrigerated overnight. In the morning, the samples were warmed to room temperature, washed into 250 ml volumetric flasks and NSI measured as before.

e. Experiment 5

At the same time experiment 4 was conducted, a separate experiment was carried out to investigate the effects of treatment of the dry soybean flour with ammonia gas on the NSI. The absorption of ammonia gas by casein and other dry isoelectric proteins has been described as an energy conserving process for producing soluble proteins for industrial food applications (367).

Ammoniated soybean flour was prepared by placing 3000 g batches under vacuum in a chamber (31.25 cm x 27.5 cm) connected to a water aspirator. After 10-15 min, the pump was closed off and dry ammonia gas was admitted so that a slightly reduced pressure was maintained over a period of about 1 hour. The treated soybean flour was then heated to 60°C and degassed under vacuum for 1 hr to remove excess ammonia from the flour particles. After degassing, the flour contained 0.60% additional nitrogen due to residual adsorbed ammonia.

329 g of ammoniated soy flour was then mixed into 5671 ml of water or whey (6.56% total solids) heated to 90°F. From this point, the samples were processed and analyzed as described in experiment 4. pH of the aqueous solution of ammoniated soy flour was 9.22; pH decreased to 8.15 when the ammoniated soybean flour was dispersed in whey.
2. Ultracentrifugation

To evaluate the effect of homogenization on the soybean proteins in the toasted defatted flour, an ultracentrifugal analytical technique was used as follows:

Toasted defatted Nutrisoy (NSI 16.7%) for ultracentrifuge analysis was slurred in distilled water (200 g in 4 liters) and stirred for 30 min. The sample was recirculated in the homogenizer and a sample was drawn for a control. The remaining material was homogenized double stage at 211.0-35.2 kg/cm$^2$ (3000-500 psi). Both samples were permitted to settle by gravity at room temperature for 1 hour.

The supernatants were collected; about 10 ml of each sample was dialyzed overnight against standard buffer (77) composed of 0.0325M K$_2$HPO$_4$, 0.0026M KH$_2$PO$_4$, 0.4M NaCl, pH 7.6, ionic strength 0.5, and containing 0.01M 2-mercaptoethanol. A final concentration of 2 to 3% protein was obtained.

Sedimentation velocities were measured with a Beckman Spinco Model E analytical ultracentrifuge. The cell, with a single sector epon filled charcoal centerpiece, was filled with sample, inserted into the instrument and allowed to equilibrate to 20°C.

All runs were carried out at 48,000 rpm and 20°C. The analyzer bar angle was set at 65° initially; pictures were taken every 16 minutes on Kodak metallographic plates with an exposure time of 8 seconds. The bar angle was 50°.

Upon completion of each run, the Svedberg coefficients were calculated for each peak.
The area under the curve of each peak was measured by cutting out a projection of each peak and weighing it. In this way, it was possible to calculate the percentage of the total area contributed by each peak.

3. Effect of 2-mercaptoethanol treatment on settling

Effects of treatment with 2-mercaptoethanol on the settling characteristics of WSDM were evaluated. Powders used were the six powders prepared for confirmation of the RSM predictions because they were fresh samples. In addition, the WSDM sample commercially prepared by the Sanna Division of Beatrice Foods was examined, even though old, because of its unusually low free fat content.

For the settling test, powders were reconstituted to 15% total solids; 100 g aliquots were weighed into 250 ml beakers and treated with 2-mercaptoethanol (10 m moles/l). A second set of untreated samples was weighed out for controls.

After holding for 3 hours at room temperature, all samples were capped and stored undisturbed in the cold for 24 hours. Phase separation was then measured as previously described.

The supernatants of the settled samples were carefully aspirated off and analyzed for total solids, total lipid and total protein.

Before storage in the cold, specific gravity measurements were made on all samples at room temperature. In addition, de (by Coulter Counter) was measured on one sample (Set I, Sample 2) that had been treated with 2-mercaptoethanol.
4. Effect of Ca$^{++}$ on NSI

The effect of calcium ion (Ca$^{++}$) on the NSI of defatted soy flour was examined as follows:

A stock solution containing 400 mg Ca$^{++}$/l was prepared with CaCl$_2$·2H$_2$O and neutralized with 5N sodium hydroxide. Solutions containing 50 and 200 mg Ca$^{++}$/l were prepared from the stock solutions. The solutions plus a control containing no calcium were heated to 33°C and defatted flour added so as to have 5.48% total solids from the flour. The samples were held 1 hr at 33°C after which the sample temperature was raised to 43°C, held for 30 min and cooled to 25°C. The pH was read; it was necessary to adjust the pH of the samples containing 200 and 400 mg Ca$^{++}$/l back to 6.65 in spite of having neutralized the Ca$^{++}$ solution used. 100 g aliquots were weighed into 250 ml volumetric flasks and NSI was measured as before. The flour used in this experiment was a moderately toasted one.
RESULTS

A. Evaluation of Commercially Prepared WSDM Powders

Partial compositional analyses and properties related to the re-hydration of four WSDM samples prepared commercially under specifications (159) are listed in Table 14. Samples A and B were prepared with full fat flour, and C and D, defatted flour. All samples contained added vitamins and minerals.

All samples met specifications for composition except Sample A which was slightly high in moisture (specifications: 3.25%). Sample C, containing the least amount of free fat had the best dispersibility and the least amount of phase separation on standing.

Sample B was less stable than the other three samples as far as phase separation and solubility characteristics (NSI and ADMI solubility index) were concerned. This sample also showed 0.4 of a unit lower pH than any of the other samples.

For examination of the composition of the supernatant liquids, the four samples were reconstituted to 15% total solids and permitted to whey off. Ratios of the total solids, protein and fat present in the supernatants to the total solids, protein and fat in the well-mixed samples are listed in Table 15. Sample B, which exhibited greatest phase separation on standing, contained the least amount of fat, protein and total solids in the supernatant. The fat ratio for
Table 14. Properties of Commercially Prepared WSDM Powders

<table>
<thead>
<tr>
<th></th>
<th>Full Fat Flour</th>
<th>Defatted Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Moisture %</td>
<td>3.60</td>
<td>3.04</td>
</tr>
<tr>
<td>Total fat %</td>
<td>21.19</td>
<td>20.77</td>
</tr>
<tr>
<td>Total protein %</td>
<td>20.73</td>
<td>22.33</td>
</tr>
<tr>
<td>(Total N x 6.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of total that is</td>
<td>16.4</td>
<td>10.2</td>
</tr>
<tr>
<td>free fat %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen solubility</td>
<td>53.3</td>
<td>29.6</td>
</tr>
<tr>
<td>index %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of total fat in</td>
<td>77.9</td>
<td>40.6</td>
</tr>
<tr>
<td>soluble nitrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADMT solubility</td>
<td>4.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Index ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinkability %</td>
<td>81.8</td>
<td>88.6</td>
</tr>
<tr>
<td>Dispersibility %</td>
<td>81.7</td>
<td>96.5</td>
</tr>
<tr>
<td>Bulk density g/cc</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>Phase separation</td>
<td>0.2</td>
<td>3.75</td>
</tr>
<tr>
<td>1 hr., ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase separation</td>
<td>37.5</td>
<td>52.9</td>
</tr>
<tr>
<td>24 hr., %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (15% TS)</td>
<td>6.59</td>
<td>5.92</td>
</tr>
</tbody>
</table>
Table 15. Composition of Wheyed-Off Supernatants of Commercial Samples

<table>
<thead>
<tr>
<th>Ratio X 100</th>
<th>Full Fat Flour</th>
<th>Defatted Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Total solids</td>
<td>65.7</td>
<td>58.3</td>
</tr>
<tr>
<td>15% Total solids</td>
<td>36.5</td>
<td>21.1</td>
</tr>
<tr>
<td>Protein</td>
<td>11.6</td>
<td>0.84</td>
</tr>
<tr>
<td>Total fat</td>
<td>11.6</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Sample C was also considerably lower than those for Samples A and D.

Flow curves generated from Brookfield viscosity measurements for the four samples reconstituted to 15% total solids are plotted in Figure 9. The shapes of the curves indicate that the shear stress-shear rate behavior was non-Newtonian and similar to that of a pseudo-plastic material. Greatest deviation from Newtonian behavior was shown by Sample B.

Average particle diameters with respect to number, linear dimension, surface area and volume for the four WSDM powders are listed in Table 16. Data were calculated as shown by the example given in Appendix A. Results indicate that the largest particles were found in Sample D. During microscopic examinations, it was apparent that this sample not only contained many aggregates measured as one particle but also contained more particles greater than 100 micrometers in diameter than did the other samples; this accounts for the elevated value for $\bar{d}_3$, the geometric mean on a weight basis, the value of which is readily skewed upward by a few very large particles. $\bar{D}$, the diameter based on the average volume per particle, was not appreciably higher than that for sample B, however.

The effect of large particles on the value of the geometric mean may also be shown graphically. Log probability plots of particle size distribution by weight for the four samples are shown in Figures 10, 11, 12 and 13. The 50% value of the distribution is equal to the geometric median diameter by weight, $\bar{d}_3$. Because the data for the four samples are curves, the distributions are not log-normal. In addition, the shape of the curves for sample B and C (Figures 11 and
Viscosity flow curves for commercially prepared WSFM powders.

Figure 9
Table 16. Average Particle Diameter of Commercially Prepared WSDM Powders (2 Micrometer Intervals)

<table>
<thead>
<tr>
<th></th>
<th>Full Fat Flour</th>
<th>Defatted Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>d1</td>
<td>21.81</td>
<td>27.50</td>
</tr>
<tr>
<td>d2</td>
<td>34.53</td>
<td>42.86</td>
</tr>
<tr>
<td>d3</td>
<td>48.92</td>
<td>60.27</td>
</tr>
<tr>
<td>d4</td>
<td>61.77</td>
<td>78.99</td>
</tr>
<tr>
<td>Δ</td>
<td>27.44</td>
<td>34.33</td>
</tr>
<tr>
<td>D</td>
<td>33.27</td>
<td>41.39</td>
</tr>
</tbody>
</table>
Log probability plot of particle size distribution of commercial sample A.

Figure 10
Log probability plot of particle size distribution of commercial sample B.
Log probability plot of particle size distribution of commercial sample C.

Figure 12
Log probability plot of particle size distribution of commercial sample D.

Figure 13
12) indicate a clear bimodality in which the experimental points asymptote toward the original parent distribution with the smaller geometric mean ($\bar{d}_H$). The shape of the curve for sample D (Figure 13) although not as sharply defined, also suggests a bimodal distribution. However, data for Sample A (Figure 10) show that the curve is asymptotic toward an upper size limit (particles formed are not infinitely large) (196).

Average particle diameters, as measured by Coulter Counter, of the reconstituted powders are listed in Table 17. Data were calculated as shown by the example given in Appendix B. It is immediately apparent that the average equivalent spherical diameter of the dispersed particles in Sample B is over 10 micrometers larger than the de of the other samples.

Log probability plots of the particle size distributions for the reconstituted samples are shown in Figure 14. Although not well defined, the shapes of these curves indicate that an artificial separation has taken place in that particles have apparently been excluded from measurement at both ends of the distribution. Equipment limitations prevented measurement of particles smaller than 8 micrometers in the reconstituted samples, so particles below this size, although present, were excluded from the measurements. The curves seemed to approach the bigger particle sizes asymptotically, but there was no exclusion of particles in the upper size range from the measurements.

PAG disc electrophoresis patterns of the supernatants of wheyed-off reconstituted Samples A, B and C are shown in Plate I. The pattern for the supernatant from toasted, defatted soy flour is also
Table 17. Average Particle Diameters of Commercially Prepared WSDM Samples Reconstituted to 15% Total Solids (Coulter Counter) (Particles Greater than 8 Micrometers)

<table>
<thead>
<tr>
<th></th>
<th>Full Fat Flour</th>
<th>Defatted Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>d_1</td>
<td>11.60</td>
<td>17.94</td>
</tr>
<tr>
<td>d_2</td>
<td>13.33</td>
<td>26.54</td>
</tr>
<tr>
<td>d_3</td>
<td>16.41</td>
<td>38.25</td>
</tr>
<tr>
<td>d_4</td>
<td>21.24</td>
<td>49.30</td>
</tr>
<tr>
<td>\bar{\Lambda}</td>
<td>12.44</td>
<td>21.82</td>
</tr>
<tr>
<td>(\bar{D})_{de}</td>
<td>13.64</td>
<td>26.31</td>
</tr>
</tbody>
</table>
Log probability plot of particle size distributions of dispersed particles of reconstituted commercially prepared WSDM samples.

Figure 14
Plate I. PAG disc electrophoresis patterns of supernatants of reconstituted commercially prepared samples A, B and C.

1. defatted toasted soybean flour
2. Sample A
3. Sample C
4. Sample B
5. Whey
shown, along with a typical whey pattern. There is no evidence of any soybean proteins present in the three samples. Sample B shows only a trace of β-lactoglobulin and none of the other whey proteins. Sample A shows the most concentrated pattern of whey proteins, suggesting that this sample received minimal heat treatment during product processing.

Because results with the PAG electrophoresis were so inconclusive, the same samples were examined by gel electrophoresis in a vertical starch bed; results are shown in Plate II. In addition, the supernatant from a mixture of soy flour dispersed in fresh sweet whey was analyzed. Examination of the patterns shows no bands clearly identifiable as soy protein in any of the samples containing whey. However, the slight broadening of the β-lactoglobulin band in samples A and C may be indicative of the presence of soy protein, as there is a band in that region in the soybean flour supernatant.

B. Examination of Particle Structure by Light Microscopy and Scanning Electron Microscopy (SEM)

Soybean flours used in product manufacture were examined by SEM. The general appearance of full fat and defatted soybean flours is shown in Plate III. The full fat flour contains a number of spherical or elliptical particles about 3-10 micrometers in diameter that presumably are intact protein bodies that survived processing. The toasted defatted flour shows many more irregular particles than the full fat flour, some of which appear ruptured. Protein bodies are difficult to identify with certainty in this sample. Both flours had the general appearance of the flours shown in SEM photomicrographs
Plate II. Starch gel electrophoresis patterns of supernatants of reconstituted soybean flour and commercially prepared samples A, B and C.

1. defatted toasted soybean flour control
2. defatted toasted soybean flour homogenized 211-35.2 kg/cm²
3. defatted toasted soybean flour control
4. defatted toasted soybean flour plus 2-mercaptoethanol
5. defatted toasted soybean flour plus whey
6. whey
7. Sample A
8. Sample C
9. Sample B
Plate III. SEM Photomicrographs of soybean flours.
previously published by Wolf and Baker (18).

Plate IV shows an SEM photomicrograph of an experimental sample of WSDM powder prepared with defatted flour and dried in the Dairy Products Laboratory pilot plant according to specifications (159); this is compared to a commercial sample of nonfat dry milk, the product which WSDM was designed to replace. The difference in particle size is immediately apparent, the nonfat dry milk being smaller. No attempt was made to determine particle size distribution from the SEM photomicrographs. The particles of nonfat dry milk appear spherical, dented, and show a slightly roughened surface texture compared to the smooth surface structure of WSDM. All samples of WSDM examined, whether experimental or prepared commercially, showed the same smooth surface texture. There was no detectable difference in appearance caused by using full fat or defatted flour in the formulation of the product.

SEM examination of a broken particle of the experimental sample of WSDM showed a distinct difference from nonfat dry milk. As shown in Plate V, NDM particles were hollow spheres with a shell surrounding a large central vacuole. In contrast, the WSDM particles showed a smaller hollow core surrounded by a thick shell. The fracture surface of the WSDM particle appears pitted with small holes whereas the surface of the nonfat dry milk particle is smooth, even at higher magnification. Similar pitting was observed by Buma and Henstra (368) as being typical of the fracture surfaces of whole milk powder.

The general appearance of a commercially prepared WSDM sample (Sample A) by SEM is shown in Plate VI. This sample was dried with
Plate IV. SEM photomicrographs of experimental WSDM powder compared to nonfat dry milk.
Plate V. SEM photomicrographs of broken particles of WSDM compared to nonfat dry milk.
Plate VI. SEM photomicrographs of commercially prepared sample A,
(Inlet air temperature 146.1°C)
an air inlet temperature of 146°C and concentrate total solids was 47%.
Although some individual particles are present, this powder is
characterized by many large aggregates. Aggregation apparently
occurred within the dryer; the bubbles in the crevices of the particles
suggest that water evaporation was slow due to low temperature and
high feed total solids although residence time in the dryer was
sufficient to reduce moisture content of the powder to 3.6%.

Differential staining with phosphine dye in a 1% agar solution
was combined with ultraviolet light microscopy to investigate the
first stages of dissolution of the experimental WSDM powder particles.
As shown in Plate VII, as the particle begins to dissolve, the larger
fat droplets quickly move to the surface of the particle and into the
dispersing medium. The rapid migration of fat droplets away from the
center of the dissolving particle was an effect originally observed
by Higa and Pallansch (212) with dissolving whole milk powder parti-
cles. When dispersion of the WSDM particle was complete, disengaged
fat globules were visible throughout the medium.

The appearance of the experimental sample of WSDM by SEM both
immediately after reconstitution to 15% total solids and after one
hour of standing at room temperature are shown in Plate VIII. The
very rough texture that can be seen in the initial picture indicates
that the particle has started to dissolve (in contrast to the smooth
surface of the intact powder, Plate IV). After one hour of standing,
very large particles have disappeared. Under the same conditions,
nonfat dry milk dispersed completely and showed no particles of any
kind at similar magnifications.
Plate VII. Light photomicrograph of differentially stained dissolving WSDM powder particles. Bright spots = fluorescing fat droplets.
Plate VIII. SEM photomicrographs of dissolving experimental WSDM powder particles.
After rehydration, a difference in appearance between samples prepared with full fat flour or with defatted flour could be observed. Plate IX shows an SEM photomicrograph of rehydrated particles of a commercial sample (Sample A) of WSDM prepared with full fat flour and permitted to stand for one hour at room temperature after reconstitution. The particles are irregularly shaped and under higher magnification, the particles have convoluted surfaces and appear fibrous in nature.

In contrast, as shown in Plate X, particles from a reconstituted commercial sample (Sample C) prepared with defatted flour, appear to be smaller and more nearly spherical than those observed in the sample prepared with full fat flour (Plate IX); at higher magnification, these particles appeared to be aggregates of much smaller particles. These particles bore a striking resemblance to published SEM photomicrographs of isoelectric soy protein isolates (18).

Upon differential staining of Sample C after rehydration with acridine orange and phosphine dyes and examination under ultraviolet light, the rehydrated particles had the general appearance shown in Plate XI. The particles appeared to be proteinaceous material coated with fat droplets; the convoluted surfaces observed here were similar to those observed in the SEM photomicrographs of rehydrated WSDM prepared with full fat flour (Plate IX). Not all samples examined showed the redispersed particles to be coated with fat droplets; however, a fat coated surface such as this would inhibit aggregate dispersal into water after reconstitution.
Plate IX. SEM photomicrographs of reconstituted commercially prepared sample A.
(full fat flour)
(rehydrated one hour)
Plate X. SEM photomicrographs of reconstituted commercially prepared sample C.
(defatted flour)
(rehydrated one hour)
Plate XI. Light photomicrograph of differentially stained commercially prepared sample C. Bright areas = fluorescing fat droplets.
C. Evaluation of Experimental WSDM Powders

1. Samples processed with single stage homogenization

All data for 12 powders processed at two total solids levels (12.6 and 29%) with single stage homogenization are tabulated in Appendix C. Because the results showed that none of the samples met specifications for suspension stability and moisture and free fat contents of some of the powders were excessive, it was decided to eliminate single stage homogenization as a process variable. Therefore no sample set homogenized single stage at 40% total solids was prepared and data from the 12 samples, although examined for significance by analysis of variance, were not included in the response surface analysis.

2. Samples processed with double stage homogenization

a. Analytical data

Results of the evaluations of the properties of the eighteen WSDM samples prepared with double stage homogenization at three total solids levels are tabulated in Table 18. Examination of the data show some variation in the total solids of the concentrates fed to the spray dryer for those sample sets homogenized at 13.7% and 26% total solids; because the samples of the third set were standardized to 40% homogenization after condensing, total solids variation in this set was minor.

The moisture contents of the first two samples of the set homogenized at 13.7% total solids were slightly higher than that permitted by the specifications (3.25%) (159). However, the values were no higher than that found for the commercially prepared sample A
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*E = 0.5% Emulsifier Added.
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*E = 0.5% Emulsifier Added.
(Table 14). Analysis for protein content showed that all samples contained amounts well above the minimum permitted by specifications (19.0%) (159). However, the fat content of six of the samples was below that allowed (19.0%) and reflected poor mixing of the fluid blends upon dividing into thirds for homogenization. The experiment should have been repeated at this point, but it was impossible to do so because of operating difficulties in the USDA-ERRC pilot plant.

Use of emulsifier in the formulation resulted in decreased NSI over the controls in all three sample sets. NSI also increased with increasing homogenization pressure, regardless of the presence of emulsifier. Highest levels of soluble nitrogen, 49.4% for the control and 47.3% for the sample containing emulsifier, were found in samples homogenized at 40% total solids and 211.0-35.2 kg/cm².

The free fat content of the powders was doubled, on the average, in all samples containing emulsifier. Least free fat was found in the samples homogenized at 40% total solids and 211.0-35.2 kg/cm². Double stage homogenization also resulted in lower free fat contents in the sample sets homogenized at 13.7 and 26% total solids, compared to the samples homogenized single stage (see Appendix C).

The fat contents of the soluble nitrogen fractions were measured to gain some information as to whether lipid was adsorbed to the dispersed insoluble material removed during the centrifugation step of the NSI procedure. The data show that although the bulk of the lipid was found in the supernatant fraction, there did seem to be a decrease with increasing homogenization pressure. The samples containing emulsifier also appeared to have more lipid in the supernatant.
fraction than the controls.

The ADM solubility index was measured on all samples. Results show that the solubility index decreased slightly with increasing homogenization pressure in all sample sets. Although the presence of the emulsifier did not alter the solubility in the samples homogenized at 13.7% total solids, solubility index was slightly higher in the emulsifier containing samples of the sets homogenized at 26% and 40% total solids, indicating that the presence of emulsifier decreased the solubility of samples homogenized at the same pressures.

Bulk densities varied very little; highest bulk densities were found in powders homogenized at 40% total solids during processing. All values conformed to density requirements (0.32 to 0.53 g/cc) in the specifications, however (159).

Sinkability was used as a measure of the wettability of the powders. All samples containing added emulsifier showed increased sinkability; values ranged from 86 to 100% compared to values of 30 to 84% for controls, suggesting that emulsifier increased the wettability of the powder particles. This finding agreed with results reported by Nelson and Winder (260) who demonstrated that the sinkability of whole milk powder was considerably improved by the addition of surface-active agents before drying.

Even though the powders with emulsifier sank better, dispersibility was reduced. Dispersibility values for the emulsifier containing samples homogenized at 40% total solids at pressures of 126.6 and 175.8 kg/cm² were close to values reported by Tamsma and Sutton (264) for spray dried whole milk powders; however, all other
values were undesirably low. Dispersibilities of the control samples were excellent, however. The increased free fat contents in the emulsifier containing samples were probably responsible for the reduced dispersibilities observed, since free fat has been associated with poor dispersing properties of whole milk powders (221, 222, 223).

When suspension stability was measured after reconstitution to 15% total solids and standing for one hour, results showed that some of the samples homogenized at 126.6-35.2 kg/cm² did not meet specifications (159). Specifications require that the supernatant liquid not exceed 10 ml. Greatest settling was observed in the samples homogenized at 26% total solids; in this sample set, the presence of emulsifier increased the settling.

After 24 hours of standing in the cold after reconstitution, settling was greatest in those samples containing added emulsifier and homogenized at 40% total solids and at pressures of 126.6-35.2 and 175.8-35.2 kg/cm². There was little difference in suspension stability among the controls of the three sample sets homogenized at the lower two pressures although stability improved with increased homogenization pressure. Best suspension stability was found in the sample homogenized at 211.0-35.2 kg/cm²; suspension stability also improved with increasing total solids of homogenization. These results suggest that raising the homogenization pressure further should effect a significant improvement in suspension stability; however, practical considerations may limit the use of higher pressures.

The results of pH measurements indicate that pH variations could be eliminated as a factor that could affect the solubility
measurements.

Flow curves were generated from the Brookfield viscosity measurements made on the reconstituted powders; these curves are shown in Figures 15, 16, 17, 18, 19 and 20. The shapes of the curves are similar to those obtained for the commercial powders (Figure 9) and indicate that the shear stress-shear rate behavior was non-Newtonian in nature and similar to that of a pseudo-plastic material.

Comparison of the curves for the samples prepared with emulsifier show that the viscosity increased both with increased homogenization pressure and increased total solids of homogenization. Viscosities of the samples containing emulsifier were lower than those of the controls in all cases. Although viscosity increased with increasing homogenization pressure, the increases were not as great as in the controls. In addition, with the exception of the sample homogenized at 211.0-35.2 kg/cm$^2$ and 40% total solids, viscosities of the emulsifier-containing samples from the three sample sets were all about the same as those of the controls homogenized at 13.7% total solids, indicating that the total solids of homogenization had little effect on viscosity increase in the presence of emulsifier.

A summary of the average particle diameters of the 18 powder samples is tabulated in Table 19. Data were calculated as shown in the example given in Appendix A. Examination of the results shows considerable variation in the data, especially in the geometric means ($\bar{d}_h$). Some of the variation in $\bar{d}_h$ may be due to differences in the total solids of the concentrates fed to the spray dryer in the sample sets homogenized at 13.7 and 26% total solids; this is known
A = 126.6-35.2 kg/cm²  B = 175.8-35.2 kg/cm²
C = 211.0-35.2 kg/cm²

Viscosity flow curves for reconstituted powders homogenized
double stage at 13.7% total solids.

Figure 15
Viscosity flow curves for reconstituted powders homogenized double stage at 13.7% total solids with 0.5% emulsifier.

Figure 16
$A = 126.6 - 35.2 \text{ kg/cm}^2 \quad B = 175.8 - 35.2 \text{ kg/cm}^2 \quad C = 211.0 - 35.2 \text{ kg/cm}^2$

Shear stress vs. shear rate plot for reconstituted powders homogenized double stage at 26% total solids.

Figure 17
A = 126.6-35.2 kg/cm²  B = 175.8-35.2 kg/cm²  C = 211.0-35.2 kg/cm²

Viscosity flow curves for reconstituted powders homogenized double stage at 26.0% total solids with 0.5% emulsifier.

Figure 18
A = 126.6-35.2 kg/cm²  B = 175.8-35.2 kg/cm²  C = 211.0-35.2 kg/cm²

Viscosity flow curves for reconstituted powders homogenized double stage at 40.0% total solids.

Figure 19
A = 126.6 - 35.2 kg/cm²  B = 175.8 - 35.2 kg/cm²  C = 211.0 - 35.2 kg/cm²

Viscosity flow curves for reconstituted powders homogenized double stage at 40.0% total solids with 0.5% emulsifier.

Figure 20
Table 19. Average Particle Diameters of Experimental WSDM Powder Samples Homogenized Double Stage at 13.7, 26.0 and 40.0% Total Solids

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>d₁</th>
<th>d₂</th>
<th>d₃</th>
<th>d₄</th>
<th>Δ</th>
<th>D</th>
</tr>
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<tr>
<td>Second Stage 35.2 kg/cm²</td>
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<tr>
<td>126.6</td>
<td>12.48</td>
<td>22.41</td>
<td>36.64</td>
<td>51.59</td>
<td>16.72</td>
<td>21.72</td>
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<tr>
<td>175.8</td>
<td>14.73</td>
<td>23.79</td>
<td>37.61</td>
<td>58.65</td>
<td>13.75</td>
<td>23.65</td>
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<td>211.0</td>
<td>16.24</td>
<td>25.90</td>
<td>38.83</td>
<td>53.82</td>
<td>20.51</td>
<td>25.37</td>
</tr>
<tr>
<td>126.6E*</td>
<td>16.40</td>
<td>25.79</td>
<td>34.71</td>
<td>41.39</td>
<td>20.56</td>
<td>24.48</td>
</tr>
<tr>
<td>175.8E</td>
<td>14.30</td>
<td>20.03</td>
<td>28.52</td>
<td>40.81</td>
<td>16.92</td>
<td>20.14</td>
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<td>37.16</td>
<td>19.42</td>
<td>22.32</td>
</tr>
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<td>37.14</td>
<td>21.73</td>
<td>24.61</td>
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<td>18.18</td>
<td>24.25</td>
<td>31.07</td>
<td>37.75</td>
<td>21.00</td>
<td>23.93</td>
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<td>175.8E</td>
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<td>22.06</td>
<td>27.46</td>
<td>32.43</td>
<td>19.01</td>
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<td>16.59</td>
<td>19.60</td>
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<tr>
<td>175.8</td>
<td>13.88</td>
<td>23.45</td>
<td>34.01</td>
<td>44.00</td>
<td>18.04</td>
<td>22.28</td>
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<tr>
<td>211.0</td>
<td>13.97</td>
<td>20.85</td>
<td>27.26</td>
<td>33.04</td>
<td>17.06</td>
<td>19.95</td>
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<td>13.46</td>
<td>17.96</td>
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<td>16.30</td>
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<tr>
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<td>12.45</td>
<td>18.04</td>
<td>24.00</td>
<td>29.63</td>
<td>14.98</td>
<td>17.53</td>
</tr>
</tbody>
</table>

*E = 0.5% Emulsifier Added
to alter the particle size distribution in milk powders (197). This does not explain the variation in $d_4$ observed in the samples homogenized and dried at 40% total solids. However, the value of $d_4$ is readily skewed by the inclusion of one or two very large particles in the measurements; examination of the raw data for this sample set showed this to be the case for the sample pair homogenized at 175.8-35.2 kg/cm$^2$, both of which had higher values of $d_4$ than the other samples in the set. An increase in the number of particles measured would yield a more accurate distribution.

Average volume particle diameters ($D$) showed much less variation within sample sets; average diameters ranged from 17.5 to 25.4 micrometers. The smallest particles, on the average, were found in the sample set homogenized at 40% total solids.

Log probability plots of the particle size distributions on a weight basis ($\mu m^3$) of all the samples were prepared. The majority of the plots had the general form shown in Figure 21, representing data for the sample homogenized without emulsifier at 26% total solids and 211.0-35.2 kg/cm$^2$. The shape of the curve resembles that found for the commercially prepared sample A (Figure 10).

Three of the curves (homogenized at 13.7% total solids and 175.8-35.2 kg/cm$^2$ both with and without emulsifier and at 40% total solids and 211.0-35.2 kg/cm$^2$ without emulsifier) had the general appearance shown in Figure 22 for data for the sample homogenized at 13.7% total solids and 175.8-35.2 kg/cm$^2$ without emulsifier. This curve resembled those of the commercially prepared samples B and C (Figures 11 and 12).
Log probability plot of particle size distribution of experimental WSDM powder homogenized at 211.0-35.2 kg/cm² and 26.0% total solids.

Figure 21
Log probability plot of particle size distribution of experimental WSDM powder homogenized at 175.8-35.2 kg/cm² and 13.7% total solids.

Figure 22
Because the plots of Figures 21 and 22 are non-linear, the data apparently follow a modified log normal distribution. The bulk of the curves, such as the one in Figure 21, were asymptotic toward an upper limit size, a modification frequently due to variables such as the orifice size controlling the formation of the powder particles (196). The general shape of the curve in Figure 22 indicates a definite bimodality in the distribution which could be caused by powder aggregation in the dryer after atomization or by agglomeration after drying, resulting in a few very large particles.

The average equivalent spherical diameters (de) for the dispersed particles of the 18 reconstituted powder samples are tabulated in Table 20. Data are given in micrometers for the de's above both 3 and 10 micrometers and for the weight per cent. The data were calculated as shown by the example given in Appendix B.

Examination of the data show that the presence of emulsifier appeared to reduce the average particle diameter when samples were homogenized at the same pressures; this can be clearly seen from the weight per cent data for the three sample sets. Increasing homogenization pressure also appeared to reduce the particle size in the samples containing emulsifier.

de of the sample homogenized without emulsifier at 13.7% total solids and 211.0-35.2 kg/cm$^2$ was higher than de's for all of the other samples. This is probably a result of the poor mixing of the fluid ingredients during processing that was apparent when the composition of the samples of this set (homogenized at 13.7% total solids) was considered (Table 18).
Table 20. Average Equivalent Spherical Diameter de and Weight Percentages of WSDM Powders Reconstituted to 15% TS

<table>
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<tr>
<th>Homogenization Pressure</th>
<th>Second Stage Pressure</th>
<th>de &gt; 8 micrometers</th>
<th>de &gt; 10 micrometers</th>
<th>Cumulative Weight Per Cent</th>
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<tr>
<td></td>
<td></td>
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<td>13.7% TS Homogenized</td>
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<tr>
<td>126.6</td>
<td>12.79</td>
<td>14.71</td>
<td>84.5</td>
<td></td>
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<td>175.8</td>
<td>12.24</td>
<td>13.99</td>
<td>82.0</td>
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<td>211.0</td>
<td>15.10</td>
<td>16.82</td>
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<td>26.0% TS Homogenized</td>
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<td>14.15</td>
<td>84.0</td>
<td></td>
</tr>
</tbody>
</table>

*E = 0.5% Emulsifier Added
Log probability plots were prepared for the particle size distribution of the 18 reconstituted samples; an example is shown in Figure 23 for samples homogenized both with and without emulsifier at 13.7% total solids and 175.8-35.2 kg/cm². In contrast to data for the commercially prepared samples A, B, and C (Figure 14) many of the curves for these samples were very close to being linear in form, especially those samples containing emulsifier, an indication that the distribution is log normal.

b. Statistical interactions

1. Analysis of variance

The effects of the three processing variables on some of the 18 WSDM samples were evaluated for significance by analysis of variance (ANOVA). Properties analyzed were the moisture content of the powders, the sinkability, dispersibility, NSI, free fat content, % fat in the soluble nitrogen fraction, settling after both one hour and 24 hours of standing after reconstitution, the Brookfield viscosity at 30 rpm, the average volume particle diameter \( \overline{D} \) of the powders and the average equivalent spherical diameter \( d_e \) of the reconstituted samples. The results are tabulated in Table 21.

Examination of the results showed that the greatest effects were brought about by the presence of emulsifier. Emulsifier had a significant effect \( p < .02 \) on 3 of the 10 properties examined; the only exceptions were the moisture content and the average volume particle diameter \( \overline{D} \).

Statistical analysis showed that none of the three processing variables had a significant effect at \( p < .05 \) on the
Log probability plots of particle size distributions for dispersed particles of experimental WSDM powders homogenized at 13.7% total solids & 175.8-35.2 kg/cm².

Figure 23
Table 21. ANOVA for WSDM Powder Samples Subjected to Double Stage Homogenization

A = TS  B = Emulsifier  C = HP  AB = TS.Emulsifier
AC = TS.HP  BC = Emulsifier.HP

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Sig. Level</th>
</tr>
</thead>
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<td>1. Moisture</td>
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<tr>
<td>Total</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
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<td>.488</td>
<td>.244</td>
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<td>1.422</td>
<td>4.719</td>
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<tr>
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<td>.356</td>
<td>.178</td>
<td>.591</td>
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<td>2. Sinkability</td>
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<tr>
<td>3. Dispersion</td>
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<td>4. NSI</td>
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<tr>
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<tr>
<td>5. Free Fat</td>
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</tr>
<tr>
<td>Total</td>
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<td>1922.957</td>
<td>338.555</td>
<td>10.243</td>
<td>.0025**</td>
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<td>811.911</td>
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</tr>
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</tr>
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</table>
Table 21 (continued)

6. % Fat in Source DF SS MS F Sig. Level
   Soluble Total 17 323.922
   Nitrogen A 2 81.888 40.944 4.729 .0306*
   Fraction B 1 36.694 36.694 4.238 .0619
   C 2 101.448 50.724 5.859 .0168*
   Pooled 12 103.893 8.658

7. Settling Source DF SS MS F Sig. Level
   One Hour Total 17 644.000
   A 2 121.333 60.667 242.667 .0000***
   B 1 8.000 8.000 32.000 .0013**
   C 2 256.333 128.167 512.667 .0000***
   AB 2 36.000 18.000 72.000 .00006***
   AC 4 220.333 55.208 220.833 .0000***
   Pooled 6 1.500

8. Settling Source DF SS MS F Sig. Level
   24 Hours Total 17 2908.063
   A 2 22.754 11.377 .1395 1.0000
   B 1 644.405 644.405 7.904 .0157*
   C 2 1262.508 631.254 7.742 .0069*
   Pooled 12 978.396 81.533

9. Viscosity Source DF SS MS F Sig. Level
   Total 17 10490
   A 2 1.841 .920 8.398 .0072**
   B 1 1.869 1.869 17.052 .0020**
   C 2 3.098 1.549 14.133 .0012**
   BC 2 2.586 1.293 11.797 .0023**
   Pooled 10 1.096 .110

10. Particle Source DF SS MS F Sig. Level
    Diameter Total 17 136417
    D A 2 16.206 8.103 .896 .434
    B 1 5.876 5.876 .649 .436
    C 2 5.765 2.883 .319 .733*
    Pooled 12 106570 9.048

11. Average Source DF SS MS F Sig. Level
    Equivalent Total 17 8683
    Spherical A 2 .152 .076 .175 1.000
    Diameter B 1 3.158 3.158 7.257 .0195*
    C 2 .150 .075 .172 1.000
    Pooled 12 5.223 .435
moisture content of the dry powder. However, the actual finding of 
$p = .051$ coupled with the results of Table 18 suggest that the presence 
of emulsifier did lead to a slightly reduced moisture content in the 
powder.

All samples prepared with emulsifier showed signifi-
cantly ($p < .01$) reduced dispersibility and increased sinkability. No 
effects of homogenization pressure or of total solids of homogenization 
were observed, however.

The NSI was significantly ($p < .01$) influenced by all 
three processing variables. Although NSI was reduced by emulsifier, 
increasing homogenization pressure increased NSI, regardless of added 
emulsifier. The total solids of homogenization had a slightly differ-
ent effect; lowest levels of soluble nitrogen, on the average, were 
found in those samples homogenized at 26% total solids.

Free fat content was significantly ($p < .01$) affected 
by both emulsifier and total solids of the homogenized mixture. The 
presence of emulsifier increased the free fat content but free fat de-
creased with increasing total solids of homogenization. Homogeniza-
tion pressure seemed to have no significant effect on free fat con-
tent, however.

Both homogenization pressure and total solids of the 
mixture homogenized significantly ($p < .05$) affected the amount of fat 
found in the soluble nitrogen fraction. Greatest amounts of fat were 
found in the samples homogenized at 26% total solids; fat content de-
creased with increasing homogenization pressure. Although examination 
of the data in Table 18 suggest that greater amounts of fat were found
in samples containing emulsifier, this was not confirmed statistically.

The effect of emulsifier on the amount of phase separation was significant after both one hour (p < .01) and 24 hours (p < .02) of standing. However, the data of Table 18 show that the results are contradictory; after one hour of standing, the samples with emulsifier show less settling, but after 24 hours, they show more settling.

Homogenization pressure and total solids of the homogenized mixture both had a significant (p < .01) effect on settling after one hour of standing; however, after 24 hours, only the homogenization pressure significantly (p < .01) reduced settling. Significant (p < .01) interaction effects AB (total solids of homogenization·emulsifier) and AC (total solids of homogenization·homogenization pressure) were also observed for the phase separation after one hour of standing; however, no such effects were found after 24 hours of standing. The interaction effects observed may be explained by examination of the data in Table 18 that show that the settling after one hour of standing in the samples homogenized at 26\% total solids was greater in the samples containing emulsifier than in the controls; the reverse was true for the other two sample sets. In addition, although settling decreased with increasing homogenization pressure, settling was still greater in the samples homogenized at 175.3-35.2 kg/cm² and 211.0-35.2 kg/cm² in this sample set than in the other two sets.

Viscosity was significantly (p < .01) affected by all three processing parameters. The Brookfield viscosity values at 30 rpm used for ANOVA are listed in Table 22. An interaction effect BC
Table 22. Brookfield Viscosities of Reconstituted WSDM Powders Measured at 30 rpm

<table>
<thead>
<tr>
<th>Homogenization</th>
<th>Pressure</th>
<th>Brookfield viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/cm²</td>
<td></td>
</tr>
<tr>
<td>Second Stage</td>
<td>35.2 kg/cm²</td>
<td></td>
</tr>
</tbody>
</table>

13.7% Total solids homogenized

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>126.6</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>175.8</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>211.0</td>
<td>3.58</td>
</tr>
<tr>
<td></td>
<td>126.6E*</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>175.8E</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>211.0E</td>
<td>3.73</td>
</tr>
</tbody>
</table>

26% Total solids homogenized

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>126.6</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>175.8</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>211.0</td>
<td>4.28</td>
</tr>
<tr>
<td></td>
<td>126.6E*</td>
<td>3.08</td>
</tr>
<tr>
<td></td>
<td>175.8E</td>
<td>3.24</td>
</tr>
<tr>
<td></td>
<td>211.0E</td>
<td>3.48</td>
</tr>
</tbody>
</table>

40% Total solids homogenized

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>126.6</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>175.8</td>
<td>5.29</td>
</tr>
<tr>
<td></td>
<td>211.0</td>
<td>6.06</td>
</tr>
<tr>
<td></td>
<td>126.6E*</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>175.8E</td>
<td>3.37</td>
</tr>
<tr>
<td></td>
<td>211.0E</td>
<td>3.91</td>
</tr>
</tbody>
</table>

*E = 0.5% Emulsifier Added
(emulsifier·homogenization pressure) was also observed; samples containing emulsifier showed a much smaller viscosity increase with increasing homogenization pressure than did the controls.

None of the processing variables had a significant effect on the average volume particle diameter $D$; this might be expected from the range of total solids of the concentrates fed to the spray dryer.

The use of emulsifier resulted in a significantly ($p < .02$) smaller average equivalent spherical diameter $d_e$; $d_e$ was not significantly affected by either of the two other processing variables. This finding might account for the contradictory settling data; because the particles are smaller in the samples with emulsifier, they would settle more slowly during a one hour period of observation. However, settling was greater in the emulsifier containing samples after 24 hours, indicating that other factors besides the particle size must be considered when drawing conclusions about suspension stability.

ANOVA was not carried out either on the bulk density or the ADMI solubility index results. (The test used to measure bulk density is crude and variations among samples could just as easily be due to error in the determinations as to variation in the drying parameters.) As both the ADMI solubility index and the NSI are measures of solubility, only the NSI was examined because it was the more accurate of the two tests; results of the ADMI solubility index seemed to parallel the NSI results in that solubility was reduced in the presence of emulsifier and solubility improved with increasing
homogenization pressure regardless of added emulsifier.

2. Simple linear correlations

Some of the data were subjected to least squares analysis to determine if the properties selected had a simple linear relationship to other properties. Linear regressions of y on x were calculated. The linear correlation coefficients (r) and their degree of significance (p) are listed in Table 23.

Examination of the literature indicated that both the free fat content and the sinkability of milk powders were related to the moisture content. Therefore, both of these properties were investigated for their dependency on the moisture content; only data for the samples without emulsifier were examined because the results (Table 18) showed that the presence of emulsifier drastically increased both free fat content and powder sinkability. The correlation coefficient for the data for the free fat content of the 9 control samples showed that there was no relation to moisture content in this case; however, there was a significant (p < .05) linear relationship between moisture content and sinkability in that sinkability increased as the moisture content of the powder increased.

The literature survey also indicated that the average volume particle diameter \( \bar{D} \) was related to the total solids of the concentrate fed to the spray dryer. However, the linear correlation coefficient .346 obtained upon analyzing data for the 18 WSDM samples was not significant.

Dispersibilities of the 18 WSDM samples were examined for their relationship to the average particle diameter with respect
Table 23. Linear Correlation Coefficients

<table>
<thead>
<tr>
<th>variable</th>
<th>number of samples</th>
<th>linear correlation coefficient</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. free fat</td>
<td>9</td>
<td>.174</td>
<td>ns</td>
</tr>
<tr>
<td>2. sinkability</td>
<td>9</td>
<td>.739</td>
<td>.05</td>
</tr>
<tr>
<td>3. ( \overline{D} )</td>
<td>18</td>
<td>.346</td>
<td>ns</td>
</tr>
<tr>
<td>4. dispersibility</td>
<td>18</td>
<td>.196</td>
<td>ns</td>
</tr>
<tr>
<td>5. dispersibility</td>
<td>18</td>
<td>.527</td>
<td>.05</td>
</tr>
<tr>
<td>6. settling (24 hr)</td>
<td>18</td>
<td>-.484</td>
<td>.05</td>
</tr>
<tr>
<td>7. settling (1 hr)</td>
<td>18</td>
<td>.0001</td>
<td>ns</td>
</tr>
<tr>
<td>8. settling (24 hr)</td>
<td>18</td>
<td>.655</td>
<td>.05</td>
</tr>
<tr>
<td>9. settling (1 hr)</td>
<td>18</td>
<td>-.434</td>
<td>ns</td>
</tr>
</tbody>
</table>
to external surface ($\bar{A}$); again, no significant linear correlation was found. However, dispersibility was significantly ($p < .05$) related to the average equivalent spherical diameter $d_e$ of the reconstituted powder. The positive correlation coefficient showed that powders with smaller dispersed particles had lower dispersibilities. This effect was undoubtedly brought about by the presence of emulsifier, however.

Settling after 24 hours of standing after reconstitution was significantly ($p < .05$) related to the average equivalent spherical diameter $d_e$; settling was also significantly ($p < .05$) related to the viscosity of the reconstituted powder. Settling after one hour of standing showed no such relationship to either $d_e$ or viscosity. These results, coupled with the wide variability shown in the data for settling after one hour of standing (Table 18) suggest that one hour of standing is not sufficient to judge suspension stability because rehydration of all the powder components is probably not complete in this time.

c. Additional experiments

1. Composition of wheyed-off supernatant

It was noted during the measurements of the suspension stability that some of the samples yielded a turbid layer upon wheying off instead of a clear yellow liquid. Therefore, a compositional analysis was made of the supernatants after settling; the samples selected for analysis were from the set homogenized at 40% total solids because the compositions of these powders agreed closely with one another (Table 18). The results are listed in Table 24.
Table 24. Compositional Analysis of Wheyed Off Supernatants of Powders Homogenized Double Stage at 40% Total Solids

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>% TS 15% TS</th>
<th>g protein g total protein</th>
<th>g fat g total fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second Stage 35.2 kg/cm²</td>
<td>------------</td>
<td>-------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>126.6</td>
<td>74.1</td>
<td>42.7</td>
<td>49.3</td>
</tr>
<tr>
<td>175.8</td>
<td>68.0</td>
<td>36.6</td>
<td>19.4</td>
</tr>
<tr>
<td>211.0</td>
<td>66.5</td>
<td>34.3</td>
<td>8.4</td>
</tr>
<tr>
<td>126.6E*</td>
<td>86.5</td>
<td>47.3</td>
<td>106</td>
</tr>
<tr>
<td>175.8E</td>
<td>84.9</td>
<td>47.5</td>
<td>98.3</td>
</tr>
<tr>
<td>211.0E</td>
<td>81.2</td>
<td>46.2</td>
<td>70.8</td>
</tr>
</tbody>
</table>

*E = 0.5% added emulsifier
The three samples prepared without emulsifier wheyed off as yellow liquids although the sample homogenized at 1800 psi (126.6 kg/cm\(^2\)) was slightly turbid; the samples containing emulsifier were all turbid. Examination of the fat ratios shows that the bulk of the fat in the samples with emulsifier was to be found in the supernatant; this undoubtedly accounted for the turbidity observed. The amount of fat in the supernatants of those samples without emulsifier was much less and decreased sharply with increasing homogenization pressure showing that as homogenization pressure increased, more lipid was bound to the protein. This occurred even with emulsifier, although binding was much less. There was also a decrease in the protein ratio in the samples without emulsifier, but little change in the protein in those samples containing emulsifier. The decreases in fat and protein contents are reflected in the decrease in the total solids ratios with increasing homogenization pressure.

2. Analysis of free fat

Because it had been reported (241) that the composition of the free fat extracted from whole milk powder varied from that of the total milk fat, triglyceride composition of the free fat was determined by gas chromatography of the methyl esters. Samples analyzed were the sample pair homogenized at 13.7% total solids and 175.8-35.2 kg/cm\(^2\), both with and without emulsifier. The results are tabulated in Table 25; the analysis of the original oil used in product manufacture is shown for comparison. There was no difference in composition between the total fat and free fat fractions of each sample and that of the original oil; in addition, the presence of emulsifier
Table 25. Triglyceride Composition of Total and Free Fat Fractions

<table>
<thead>
<tr>
<th>Triglyceride</th>
<th>Control Total (per cent)</th>
<th>Control Free</th>
<th>0.5% emulsifier Total</th>
<th>0.5% emulsifier Free</th>
<th>Original oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.01</td>
<td>0.003</td>
</tr>
<tr>
<td>16:0</td>
<td>8.8</td>
<td>8.7</td>
<td>9.4</td>
<td>8.7</td>
<td>8.3</td>
</tr>
<tr>
<td>18:0</td>
<td>4.3</td>
<td>4.5</td>
<td>4.7</td>
<td>4.3</td>
<td>4.0</td>
</tr>
<tr>
<td>18:1</td>
<td>46.9</td>
<td>47.5</td>
<td>47.9</td>
<td>46.9</td>
<td>47.5</td>
</tr>
<tr>
<td>18:2</td>
<td>36.8</td>
<td>35.8</td>
<td>38.7</td>
<td>37.3</td>
<td>37.0</td>
</tr>
<tr>
<td>20:0</td>
<td>2.8</td>
<td>3.1</td>
<td>3.1</td>
<td>2.7</td>
<td>3.2</td>
</tr>
</tbody>
</table>
had no effect on the free fat composition.

d. Response surface analysis

On the basis of results obtained by analysis of the 18 WSDM powders, five properties were selected for further analysis by response surface methodology. The five properties chosen were dispersibility, free fat content, NSI, average equivalent spherical diameter (de) and phase separation after 24 hours of standing after reconstitution. These properties seemed to represent the most desirable features to be controlled when considering milk powders and dehydrated soybean beverages.

Polynomial equations were constructed for the five properties according to equation 2 (see Methods) with the 13 experimental runs. The polynomial equation coefficients are given in Table 26 along with the multiple correlation coefficients R and the coefficients of determination R^2. Because only two levels, 0 and 0.5%, of emulsifier were used in this experiment, the second order term for emulsifier could not be included.

Some of the coefficients are very small and may be dropped for practical purposes. However, all terms were used whether significant or not, so as to consider all possible interactions (369).

The statistical significance of the regression equations is shown in Table 27. The significance of the F values obtained by ANOVA show that the equations explained much of the variation with respect to each dependent variable. The exception to this is the particle size de; the equation accounts for only about 49% of the variability since the multiple correlation coefficient was only .703.
<table>
<thead>
<tr>
<th></th>
<th>( b_0 )</th>
<th>( b_1 )</th>
<th>( b_2 )</th>
<th>( b_3 )</th>
<th>( b_4 )</th>
<th>( b_5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersibility</td>
<td>68.03155</td>
<td>-311.75695</td>
<td>.028966</td>
<td>-.517686</td>
<td>-.108266</td>
<td>7.38320</td>
</tr>
<tr>
<td>Free Fat</td>
<td>16.56823</td>
<td>546.915268</td>
<td>-.0281078</td>
<td>2.86384</td>
<td>-.030584</td>
<td>-7.644357</td>
</tr>
<tr>
<td>NSI</td>
<td>41.4477585</td>
<td>-95.691662</td>
<td>.00668177</td>
<td>-.62840137</td>
<td>.02614985</td>
<td>-.44356955</td>
</tr>
<tr>
<td>de</td>
<td>16.11282055</td>
<td>30.46766035</td>
<td>-.00181378</td>
<td>.03355739</td>
<td>-.01451988</td>
<td>-.44593176</td>
</tr>
<tr>
<td>Phase separation 24 hr.</td>
<td>4.20324822</td>
<td>-880.33824556</td>
<td>.06210317</td>
<td>.36447799</td>
<td>.33311927</td>
<td>11.59055118</td>
</tr>
</tbody>
</table>

Subscript 1 = Tween  
2 = HP  
3 = TS  
4 = Tween·HP  
5 = Tween·TS  
6 = HP·TS  
7 = HP·HP  
8 = TS·TS
Table 26 (continued)

<table>
<thead>
<tr>
<th></th>
<th>$b_6$</th>
<th>$b_7$</th>
<th>$b_8$</th>
<th>Multiple Correlation Coefficient</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersibility</td>
<td>-.0000918</td>
<td>-.000005</td>
<td>.011065</td>
<td>.982</td>
<td>.964575</td>
</tr>
<tr>
<td>Free Fat</td>
<td>-.0002298</td>
<td>.00000697</td>
<td>-.04731685</td>
<td>.920</td>
<td>.84718299</td>
</tr>
<tr>
<td>NSI</td>
<td>-.00000571</td>
<td>-.000008</td>
<td>.01265415</td>
<td>.880</td>
<td>.77433937</td>
</tr>
<tr>
<td>de</td>
<td>-.00000376</td>
<td>.000005</td>
<td>-.00039377</td>
<td>.703</td>
<td>.49458455</td>
</tr>
<tr>
<td>Phase separation 24 hr.</td>
<td>-.00047559</td>
<td>-.0001551</td>
<td>.01056166</td>
<td>.930</td>
<td>.86449159</td>
</tr>
</tbody>
</table>
Table 27. Analysis of Variance for the Polynomial Equations

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dispersibility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>1765.8482</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>61.8518</td>
<td>220.7310</td>
<td>30.63</td>
<td>.0001</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>1830.7000</td>
<td>7.2058</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Free fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>1629.0961</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>293.8605</td>
<td>203.6370</td>
<td>6.24</td>
<td>.0064</td>
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<tr>
<td>Total</td>
<td>17</td>
<td>1922.9566</td>
<td>32.6512</td>
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<td></td>
</tr>
<tr>
<td>3. NSI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>93.8362</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>11.2667</td>
<td>11.7295</td>
<td>9.37</td>
<td>.0015</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>105.1029</td>
<td>1.2518</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. de</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>4.2946</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>1.3886</td>
<td>1.5363</td>
<td>1.10</td>
<td>.4405</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>8.6832</td>
<td>0.4876</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Phase Separation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>8</td>
<td>2513.9958</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>394.0670</td>
<td>414.2495</td>
<td>7.18</td>
<td>.0039</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>2908.0628</td>
<td>43.7852</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Response surface contours were generated for each of the properties to aid in visualizing the combined effects of the processing variables on the dependent responses. They are shown in Figures 24 to 33. Each plot represents the response obtained as a function of total solids of the mixture homogenized and homogenization pressure at a specified emulsifier level. In each plot it is evident that there are many combinations of variables that fit on the same response curve.

A variety of interrelationships between variables are evident from the different forms of the curves such as rising and falling ridges, bulls-eyes or saddles. For example, the contours for dispersibility and free fat, both with and without emulsifier are saddle types (Figures 24, 25, 26 and 27) indicating that at each emulsifier level (0 and 0.5%) there was interaction between homogenization pressure and total solids homogenized to give a minimum-maximum or saddle type model.

In contrast, the contours for NSI (Figures 28 and 29) represent bulls-eyes. It is immediately evident that increasing homogenization pressure increases the NSI regardless of the presence of emulsifier.

The contour plots for de with and without emulsifier (Figures 30 and 31) are quite different from one another. Although Figure 30 shows rising and falling ridges, Figure 31 (with emulsifier) clearly shows that a maximum particle size may be found through a broad range of homogenization pressure and total solids combinations.
Response surface contours for dispersibility.  
(Control)

Figure 24
Response surface contours for dispersibility.
(Emulsifier)

Figure 25
Figure 26

Response surface contours for free fat.
(Control)

TOTAL SOLIDS HOMOGENIZED, %

HOMOGENIZING PRESSURE

1800 2000 2200 2400 2600 2800 3000 psi

130 150 170 190 210 kg/cm²
Response surface contours for free fat.
(Emulsifier)
Figure 27.
Response surface contours for NSI.
(Control)

Figure 28
TOTAL SOLIDS HOMOGENIZED,

Response surface contours for NSI.
(Emulsifier)

Figure 29
Response surface contours for particle size (d_e).
(Control)

Figure 30
Response surface contours for particle size ($d_e$).

(Emulsifier)

Figure 31
Figure 32

Response surface contours for phase separation after 24 hours.
(Control)
Response surface contours for phase separation after 24 hours.
(Emulsifier)

Figure 33
The plot indicates that the smaller particle sizes are to be found by homogenizing at high total solids across virtually the entire homogenization pressure range.

Both plots for settling (Figures 32 and 33) are of the saddle type. The plot of Figure 32 shows settling to be maximum at all total solids levels as long as homogenization pressure is low. The addition of emulsifier (Figure 33) alters the settling characteristics in that the plot shows a very broad region of minimum settling across the homogenization pressure range; however, both increasing total solids and increasing homogenization pressure decrease settling.

The prediction power of each of the five regression equations for the properties selected was tested in the pilot plant. A comparison of computer predicted and observed results is tabulated in Table 28.

Three points were selected for testing and were run in duplicate. The first point, Set I, was chosen so as to maximize dispersibility; de was also minimized. The second point, Set II, was chosen to yield a product that would meet specifications for processing conditions. The third point, Set III, represented the point chosen to maximize NSI and minimize free fat and settling. All samples were prepared without emulsifier because of the undesirable effects emulsifier had on the properties selected for evaluation.

The results of Table 28 show that agreement was best between predicted and observed values of NSI. Although agreement was not as good among the results for free fat and phase separation, the trends were correctly predicted. Least agreement was found in all
Table 28. Computer Predicted vs. Observed Results

<table>
<thead>
<tr>
<th>Response</th>
<th>Set I 1 Found</th>
<th>Set I 2 Predicted</th>
<th>Set II 1 Predicted</th>
<th>Set II 2 Found</th>
<th>Set III 1 Predicted</th>
<th>Set III 2 Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersibility (%)</td>
<td>84.4</td>
<td>100</td>
<td>85.6</td>
<td>100</td>
<td>91.6</td>
<td>93.0</td>
</tr>
<tr>
<td>NSI (%)</td>
<td>44.0</td>
<td>46.3</td>
<td>44.7</td>
<td>46.4</td>
<td>46.8</td>
<td>46.2</td>
</tr>
<tr>
<td>Free fat (%)</td>
<td>28.0</td>
<td>14.1</td>
<td>26.5</td>
<td>13.7</td>
<td>21.8</td>
<td>20.5</td>
</tr>
<tr>
<td>Phase Separation 24 hr standing (%)</td>
<td>69.1</td>
<td>49.4</td>
<td>69.8</td>
<td>49.6</td>
<td>23.8</td>
<td>28.0</td>
</tr>
<tr>
<td>Micro-meters</td>
<td>14.66</td>
<td>15.03</td>
<td>14.18</td>
<td>15.03</td>
<td>14.48</td>
<td>15.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

28
cases in the predictions for dispersibility even though the multiple correlation coefficient of 0.93 for the regression equation indicated that the effects of the processing variables on the dispersibility were highly significant. Evidently, drying parameters influencing powder particle size and porosity were the major factors controlling dispersibility.

Statistically the regression equation for de was not significant and this is borne out by the wide variation (almost one micrometer) in de observed.

e. Further evaluations of average equivalent spherical diameter (de)

Since statistical evaluation (Table 21) showed de of the reconstituted WSDM powders to be significantly affected only by the emulsifier, it was decided to examine the relationship of de to the three processing variables more closely by investigating the concentrates before drying. The effects of both single and double stage homogenization were examined both with and without added emulsifier; data for single stage homogenization may be found in Appendix C.

First of all, the power input to the homogenizer was measured during homogenization at the three total solids levels; the results are tabulated in Table 29. Examination of the data show little difference in power input as a result of emulsifier presence or of total solids; however, as would be expected, the power input increased with increasing homogenization pressure. The total solids of the high solids level was below 40% but was still sufficiently high to yield meaningful results.
Table 29. Power Input Required to Homogenize WSDM Concentrates Double Stage at Three Total Solids Levels

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% amps.</td>
<td>% amps.</td>
<td>% amps.</td>
</tr>
<tr>
<td>Second Stage 35.2 kg/cm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>126.6</td>
<td>13.9</td>
<td>12.4</td>
<td>28.9</td>
</tr>
<tr>
<td>175.8</td>
<td>13.7</td>
<td>13.5</td>
<td>28.5</td>
</tr>
<tr>
<td>211.0</td>
<td>13.3</td>
<td>14.5</td>
<td>29.8</td>
</tr>
<tr>
<td>126.6E*</td>
<td>13.9</td>
<td>12.4</td>
<td>28.7</td>
</tr>
<tr>
<td>175.8E</td>
<td>13.9</td>
<td>13.4</td>
<td>28.8</td>
</tr>
<tr>
<td>211.0E</td>
<td>14.0</td>
<td>14.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>

*E = 0.5% emulsifier added
Average equivalent spherical diameter de for the concentrates homogenized at the three total solids levels are tabulated in Table 30; the cumulative weight per cent above 10 micrometers is also listed. Examination of the results showed the de to be one to two micrometers smaller at all total solids levels than de reported for the WSDM powders (Table 20). The cumulative weight percentages ranged from 51 to 79%, considerably lower than the range of 82 to 93% reported for the powders, indicating that the concentrates contained considerably more particles below 10 micrometers in diameter. These results suggest that some aggregates formed during spray drying that did not redisperse upon reconstitution. Unfortunately, none of the samples in this experiment were spray dried.

The largest particles were found in the samples homogenized at 13.9% total solids without emulsifier. de appeared to decrease with increasing homogenization pressure, regardless of the presence of emulsifier. de of samples with emulsifier was about the same in samples homogenized at 13.8 or 28.8% total solids and tended to be smaller than controls; however, unlike the other sample sets, de of samples homogenized at 36.6% with emulsifier was about the same as that of the controls.

Statistical evaluation by ANOVA, listed in Table 31, confirmed that there was a significant increase in power input with increased homogenization pressure, but no other significant effects were observed. However, all three processing variables were shown to have a highly significant effect on de in this experiment; in addition, there were significant interaction effects of the total solids of the
Table 30. Average Equivalent Spherical Diameter de of Concentrates Homogenized Double Stage at Three Total Solids Levels

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>Second Stage 35.2 kg/cm²</th>
<th>de &gt; 8 micrometers</th>
<th>Cumulative Weight Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 13.8% TS Homogenized----</td>
</tr>
<tr>
<td>126.6</td>
<td>11.72</td>
<td>79.3</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td>11.31</td>
<td>74.1</td>
<td></td>
</tr>
<tr>
<td>211.0</td>
<td>11.44</td>
<td>77.0</td>
<td></td>
</tr>
<tr>
<td>126.6E*</td>
<td>10.72</td>
<td>65.4</td>
<td></td>
</tr>
<tr>
<td>175.8E</td>
<td>10.66</td>
<td>64.4</td>
<td></td>
</tr>
<tr>
<td>211.0E</td>
<td>10.45</td>
<td>60.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 28.8% TS Homogenized----</td>
</tr>
<tr>
<td>126.6</td>
<td>10.76</td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td>11.32</td>
<td>73.6</td>
<td></td>
</tr>
<tr>
<td>211.0</td>
<td>11.05</td>
<td>70.6</td>
<td></td>
</tr>
<tr>
<td>126.6E*</td>
<td>10.76</td>
<td>65.4</td>
<td></td>
</tr>
<tr>
<td>175.8E</td>
<td>10.57</td>
<td>59.6</td>
<td></td>
</tr>
<tr>
<td>211.0E</td>
<td>10.55</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- 36.6% TS Homogenized----</td>
</tr>
<tr>
<td>126.6</td>
<td>10.96</td>
<td>64.9</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td>10.46</td>
<td>55.3</td>
<td></td>
</tr>
<tr>
<td>211.0</td>
<td>10.24</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>126.6E*</td>
<td>11.26</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td>10.74</td>
<td>60.9</td>
<td></td>
</tr>
<tr>
<td>211.0</td>
<td>10.27</td>
<td>51.1</td>
<td></td>
</tr>
</tbody>
</table>

*E = 0.5% emulsifier added
### Table 31. Analysis of Variance for WSDM Concentrates Homogenized Double Stage

A = TS  B = Emulsifier  C = HP  
AB = Emulsifier·TS  AC = TS·HP

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Sig. Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>17</td>
<td>12.230</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>.126</td>
<td>.063</td>
<td>1.699</td>
<td>.224</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>.014</td>
<td>.014</td>
<td>.375</td>
<td>.552</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>11.646</td>
<td>5.823</td>
<td>157.219</td>
<td>.0000***</td>
</tr>
<tr>
<td>Pooled</td>
<td>12</td>
<td>.444</td>
<td>.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>.013</td>
<td>.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Sig. Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>17</td>
<td>1.838</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>.337</td>
<td>.169</td>
<td>78.224</td>
<td>.00005***</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>.325</td>
<td>.325</td>
<td>150.938</td>
<td>.00002***</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>.363</td>
<td>.181</td>
<td>84.155</td>
<td>.00004***</td>
</tr>
<tr>
<td>AB</td>
<td>2</td>
<td>.517</td>
<td>.258</td>
<td>119.879</td>
<td>.00001***</td>
</tr>
<tr>
<td>AC</td>
<td>4</td>
<td>.283</td>
<td>.071</td>
<td>32.807</td>
<td>.00032***</td>
</tr>
<tr>
<td>Pooled</td>
<td>6</td>
<td>.013</td>
<td>.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>.013</td>
<td>.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
mixture homogenized both with emulsifier and with homogenization pressure.

Viscosity behavior of the concentrates was also investigated since linear regression analysis of the WSDM powders (Table 23) had shown significant relationships between phase separation and both de and viscosity.

Figures 34, 35 and 36 show the flow curves generated from Brookfield viscosity measurements of the concentrates after homogenization at the three total solids levels. Figures 37 and 38 show the flow curves found after the samples homogenized at 28.8 and 36.6% total solids were diluted back to 15% total solids. Inspection of the first three figures shows a sharp increase in viscosity with increasing total solids homogenized as would be expected. The samples containing emulsifier also exhibited lower viscosities than their controls. The flow curves of the diluted sample sets (Figures 37 and 38) differed very little from one another with the exception of the sample homogenized without emulsifier at 126.6-35.2 kg/cm² and 28% total solids; this sample had an unusually high viscosity that was probably the result of a dilution error. Both diluted sample sets had the same (36.6% total solids) or lower (28.8% total solids) viscosities as the set homogenized at 13.8% total solids. These results suggest that a gel structure built up by homogenization was broken down by dilution perhaps due to resolubilization of some of the components. Comparison of these curves to those for the WSDM powders (Figures 15-20) indicate that the gel structure is broken down further by the drying process, never to be completely reformed upon rehydration; the flow curves of
A and D = 126.6-35.2 kg/cm²
B and E = 175.8-35.2 kg/cm²
C and F = 211.0-35.2 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized at 13.8% total solids.
(Emulsifier = A, B, C)

Figure 34
A and D = 126.6 - 35.2 kg/cm²
B and E = 175.8 - 35.2 kg/cm²
C and F = 211.0 - 35.2 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized at 28.8% total solids.
(Emulsifier = A, B, C)

Figure 35
A and D = 126.6-35.2 kg/cm²  B and E = 175.8-35.2 kg/cm²  C and F = 211.0-35.2 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized at 36.6% total solids.  (Emulsifier = A, B, C)

Figure 36
A and D = 126.6-35.2 kg/cm²  B and E = 175.8-35.2 kg/cm²  C and F = 211.0-35.2 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized at 28.8% total solids
diluted to 15% total solids.
(Emulsifier = A, B, C)

Figure 37
A and D = 126.6-35.2 kg/cm²  
B and E = 175.8-35.2 kg/cm²  
C and F = 211.0-35.2 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized at 36.6% total solids diluted to 15% total solids.  
(Emulsifier = A, B, C)

Figure 38
the rehydrated powders attain only about half the shear stress of the
diluted concentrates. The shapes of the concentrate flow curves
generally resemble those found for the reconstituted powders in that
the flow was non-Newtonian and similar to a pseudoplastic type of
material.

Because the concentrates studied in the preceding experiment were not those that were spray dried to produce the 18 experimental WSDM powders, the opportunity was taken, during the processing run to check the accuracy of the RSM predictions, to examine de of the concentrates as well as the powders in order to determine how spray drying affected de. None of these samples contained added emulsifier. The results are shown in Table 32 along with de for the defatted soy flour used in powder preparation. More detailed data for de of both full fat and defatted soy flour may be found in Appendix D. Also shown is the cumulative weight percentage greater than 10 micrometers. First of all, processing up to the concentrate reduced the de about 3 micrometers when compared to the de for the flour. The results clearly show an increase in de of around one micrometer as a result of spray drying, the greatest increase being in Set III, homogenized after condensing to 40% total solids. The cumulative weight percentages above 10 micrometers in diameter emphasize the differences, with Set III showing an increase from about 75 to 96% compared to about 90% for the original flour. de's for the six concentrates are somewhat larger than those observed in the preceding experiment (Table 30), but a different lot of flour was used for product preparation.
Table 32. Comparison of Average Equivalent Spherical Diameters (de) of Concentrates with de of Their Powders After Reconstitution to 15% Total Solids

<table>
<thead>
<tr>
<th>Set</th>
<th>No. 1</th>
<th>No. 2</th>
<th>Set</th>
<th>No. 1</th>
<th>No. 2</th>
<th>Set</th>
<th>No. 1</th>
<th>No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.88</td>
<td>11.83</td>
<td>II</td>
<td>11.60</td>
<td>11.91</td>
<td>III</td>
<td>11.69</td>
<td>11.66</td>
</tr>
<tr>
<td></td>
<td>77.6</td>
<td>76.7</td>
<td></td>
<td>74.0</td>
<td>78.2</td>
<td></td>
<td>75.6</td>
<td>75.4</td>
</tr>
<tr>
<td></td>
<td>12.75</td>
<td>12.08</td>
<td></td>
<td>12.66</td>
<td>12.35</td>
<td></td>
<td>12.90</td>
<td>13.12</td>
</tr>
<tr>
<td></td>
<td>84.3</td>
<td>78.8</td>
<td></td>
<td>84.3</td>
<td>82.1</td>
<td></td>
<td>85.8</td>
<td>86.3</td>
</tr>
</tbody>
</table>

Defatted soy flour used to prepare the above

- 14.76 89.8
Log probability plots of the particle size distributions of the concentrates and their resultant powders all had the same form illustrated in Figure 39. This figure shows the results for Set II, sample No. 1. The shapes of the curves indicate a modified log normal distribution; these curves are an excellent illustration of the modification in the log normal distribution brought about by artificially removing all particles below a certain size (5 micrometers) (196) from the distribution.

Effects of spray drying on NSI were determined by measuring NSI of the concentrates and comparing the values found to the NSI's of the resultant powders. The results are listed in Table 33; NSI of the defatted soy flour used is also shown. An increase in NSI of the powders was observed in all cases, suggesting that the homogenizing effect of the spinning disc atomizer used to spray dry the samples more than offset any insolubilization of the soybean proteins brought about by the drying temperature.

Figures 40-45 show the flow curves obtained from the Brookfield viscosity measurements on the concentrates and their reconstituted powders. Figures 46 and 47 show the flow curves obtained when concentrates of Sets II and III were diluted to 15% total solids. Although originally differing widely from one another (see Figures 41 and 42) viscosities of the diluted concentrates, both homogenized during processing at 211.0-35.2 kg/cm² but at different total solids levels (21.0 and 39.6%) were about the same; both were higher than the concentrates of Set I, homogenized at 175.3-35.2 kg/cm² at a total solids of 14.8% (Figure 40). Viscosities of the corresponding
Log probability plots of particle size distributions of dispersed particles of a WSDM concentrate and its resultant powder.

Figure 39
Table 33. NSI of Experimental WSDM Concentrates and Their Resultant Powders

<table>
<thead>
<tr>
<th>Set</th>
<th>No. 1</th>
<th>No. 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Set I</td>
<td>41.6</td>
<td>42.3</td>
<td></td>
<td>44.0</td>
</tr>
<tr>
<td>Set II</td>
<td>42.5</td>
<td>44.0</td>
<td>46.8</td>
<td>45.8</td>
</tr>
<tr>
<td>Set III</td>
<td>44.8</td>
<td>45.9</td>
<td>48.1</td>
<td>47.3</td>
</tr>
<tr>
<td>Defatted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy flour</td>
<td>-</td>
<td></td>
<td></td>
<td>16.7</td>
</tr>
</tbody>
</table>

---------%--------

Concentrates  Powder
Viscosity flow curves of Set I concentrates.
A = Sample No. 1            B = Sample No. 2

Figure 40
Viscosity flow curves of Set II concentrates.

A = Sample No. 1  
B = Sample No. 2

Figure 41
Viscosity flow curves of Set III concentrates.
A = Sample No. 1    B = Sample No. 2

Figure 42
Viscosity flow curves of Set I reconstituted powders.
(A and B contain 2-mercaptoethanol)
A and C = Sample No. 1  B and D = Sample No. 2

Figure 43
Viscosity flow curves of Set II reconstituted powders.

( A and B contain 2-mercaptoethanol)

A and C = Sample No. 1  B and D = Sample No. 2

Figure 44
Viscosity flow curves of Set III reconstituted powders.
(A and B contain 2-mercaptoethanol)
A and C = Sample No. 1  B and D = Sample No. 2

Figure 45
Viscosity flow curves of Set II concentrates diluted to 15% total solids.

A = Sample No. 1  
B = Sample No. 2  

Figure 46
Viscosity flow curves of Set III concentrates diluted to 15% total solids.

A = Sample No. 1
B = Sample No. 2

Figure 47
reconstituted powders (Figures 43, 44 and 45) were greatest in Set III, homogenized at 40% total solids. Viscosities of all powder samples agreed with those found for the experimental WSDM powders. The curves showed less deviation from Newtonian behavior, however, especially in the case of Set I.

Samples of the reconstituted powders were also treated with 10 mMol/l 2-mercaptoethanol, a disulfide bond reductant, to determine what effect this would have on viscosity. The flow curves of the treated samples, also shown in Figures 43, 44 and 45, indicated that 2-mercaptoethanol decreased viscosity in all cases; the decrease was least in the samples (Set I) homogenized at low total solids (14.8%) and 175.8-35.2 kg/cm². These results show that disulfide bonds are involved in viscosity buildup; at higher total solids levels, disulfide bonds, broken by the homogenization, may have a greater opportunity to reform as intermolecular linkages, resulting in increased viscosity.

D. Solubility Studies of Soybean Flour Proteins in Whey

Because of the importance of the homogenization step in the processing of WSDM and its effects on product properties, several experiments were performed to assess more closely the influence of homogenization on the solubility of the soybean flour proteins under various conditions.

1. Effects of homogenization pressure on NSI and viscosity

Both single and double stage homogenization were examined for their effects on the NSI and viscosity of defatted soybean flour dispersed in water or sweet whey; the effects of added emulsifier were
also tested. The flour level chosen, 5.5%, was slightly higher than the level present in reconstituted WSDM; it was selected so that the total protein level was equivalent to that found in the reconstituted beverage when the flour alone was dispersed in water. For purposes of comparison, the same level was used when the flour was dispersed in whey. The emulsifier level used was 0.5%, the same as that used for preparation of the experimental WSDM powders. Total solids of the whey was 6.1-6.3%.

The results of single and double stage homogenization of soybean flour, with and without emulsifier, in water or whey, are shown in Table 34. Inspection of the results showed that, in agreement with data reported by Fukushima and Van Buren (191), presence of emulsifier reduced the solubility of the soybean proteins, especially in cheese whey. The proteins without emulsifier were also less soluble in the whey; upon subtraction of the nitrogen contributed by the whey, solubility of the soybean flour proteins before homogenization was reduced from 13.7% in water to 8.9% in whey. Subsequent homogenization also had less effect on the NSI of the proteins in whey than in water. The NSI values in whey, however, were in the same range as those reported for the experimental WSDM powders (Table 18).

Increasing homogenization pressure increased the NSI in all cases. In the samples homogenized in water, addition of the second stage did not appear to have much effect at 126.6 kg/cm² for the first stage but brought about a considerable increase in NSI at the two higher pressures. This effect was not as sharp in the samples homogenized in whey; the second stage appeared to have little effect in the
Table 34. Effects of Single and Double Stage Homogenization With and Without Emulsifier on the NSI of Soybean Flour Proteins in Water or Whey

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>Water Control</th>
<th>Emulsifier*</th>
<th>Whey Control</th>
<th>Emulsifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>18.7</td>
<td>18.5</td>
<td>34.7</td>
<td>32.2</td>
</tr>
<tr>
<td>126.6</td>
<td>47.1</td>
<td>46.2</td>
<td>42.6</td>
<td>38.2</td>
</tr>
<tr>
<td>175.8</td>
<td>53.5</td>
<td>51.3</td>
<td>45.6</td>
<td>40.8</td>
</tr>
<tr>
<td>211.0</td>
<td>54.8</td>
<td>53.0</td>
<td>47.0</td>
<td>42.1</td>
</tr>
<tr>
<td>126.6-35.2</td>
<td>48.3</td>
<td>47.2</td>
<td>43.7</td>
<td>39.7</td>
</tr>
<tr>
<td>175.8-35.2</td>
<td>56.2</td>
<td>56.7</td>
<td>44.7</td>
<td>42.2</td>
</tr>
<tr>
<td>211.0-35.2</td>
<td>60.5</td>
<td>62.4</td>
<td>47.2</td>
<td>45.7</td>
</tr>
</tbody>
</table>

* Emulsifier = 0.5% Tween 60
control samples, although there was some increase in NSI upon addition of the second stage in the samples with emulsifier.

When NSI was plotted against the total homogenization pressure, the results shown in Figures 48 and 49 were obtained. The straight line plots for the samples homogenized in whey suggest a linear dependency of the NSI on total homogenization pressure whereas the plots for the samples in water are variable.

The flow curves generated from Brookfield viscosity measurements of the soybean flour proteins in water or whey are shown in Figures 50-57. Viscosities of the unhomogenized samples are shown for comparison. Although some of the flow curves resemble those reported for the reconstituted experimental WSDM powder samples in that flow was non-Newtonian and appeared to be pseudoplastic in nature, the majority showed some curvature in the opposite direction. This is especially apparent with the samples homogenized in whey with emulsifier. This type of curve is typical of dilatant flow and in some cases some yield stress had to be overcome.

There was little difference in curves homogenized single or double stage in water; curves for the samples containing emulsifier fell virtually on top of one another and corresponded to the curves for the controls homogenized single or double stage at 126.6 kg/cm² (second stage 35.2 kg/cm²).

It should be pointed out that Brookfield viscosity measurements in coarse suspensions or slurries that tend to settle rapidly are difficult to make because of the wide gaps and low speeds of rotation. The viscosities of these flour-in-water and flour-in-whey suspensions
Variation of NSI of soybean flour homogenized in water with homogenization pressure.

Figure 48
Variation of NSI of soybean flour homogenized in whey with homogenization pressure.

Figure 49
A = Control  B = 126.6 kg/cm²  C = 175.8 kg/cm²  D = 211.0 kg/cm²

Viscosity flow curves of soybean flour homogenized single stage in water.

Figure 50.
A = Control   B = 126.6 kg/cm²   C = 175.8 kg/cm²   D = 211.0 kg/cm²

Figure 51

Viscosity flow curves of soybean flour homogenized single stage in water with 0.5% emulsifier.
A = Control  B = 126.6-35.2 kg/cm²  C = 175.8-35.2 kg/cm²  
D = 211.0-35.2 kg/cm²

Viscosity flow curves of soybean flour homogenized double stage in water.

Figure 52
A = Control  B = 126.6-35.2 kg/cm²  C = 175.8-35.2 kg/cm²  
D = 211.0-35.2 kg/cm²

Viscosity flow curves of soybean flour homogenized double stage in water with 0.5% emulsifier.

Figure 53
A = Control  B = 126.6-35.2 kg/cm²  C = 175.8-35.2 kg/cm²
D = 211.0-35.2 kg/cm²

Viscosity flow curves of soybean flour homogenized single stage in whey.

Figure 54
A = Control  B = 126.6 kg/cm²  C = 175.8 kg/cm²  
D = 211.0 kg/cm²

Shear flow curves of soybean flour homogenized single stage 
in whey with 0.5% emulsifier.

Figure 55
Viscosity flow curves of soybean flour homogenized double stage in whey.

Figure 56
A = Control  B = 126.6-35.2 kg/cm²  C = 175.8-35.2 kg/cm²
D = 211.0-35.2 kg/cm²

Viscosity flow curves of soybean flour homogenized double stage
in whey with 0.5% emulsifier.

Figure 57
were very low; at the low rpms, there may not have been sufficient movement to keep the particles in suspension, especially in the samples containing emulsifier. Therefore, it is likely that the results observed were due to experimental error, especially since no such curves were observed in the other samples.

2. Effects of pH on NSI and viscosity

The effects of pH of the sweet whey used in product manufacture on the NSI and viscosity of the soybean flour proteins after homogenization were assessed. The addition of emulsifier was omitted in these experiments so as not to obscure pH effects. Lowering pH reduces the amount of protein extracted from defatted meal in water (96); protein solubility is minimal between pH's 4 and 5. The addition of neutral salts to the system helps resolubilize the proteins, even at the isoelectric point (96).

A preliminary experiment was carried out to determine if reduction of the pH of whey by acidification with lactic acid would affect NSI. The results are shown in Table 35. Although sufficient lactic acid was added to lower whey pH by one unit, when defatted flour was added, pH was only decreased by 0.5 unit; the NSI decreased very little. Therefore it was decided to ferment the whey naturally to assess the effects of acidification on NSI.

Table 36 shows that total solids of the wheys changed very little with the development of natural acidity in the whey. However, upon addition of the soy flour prior to homogenization, the pH of the mixture was raised and the raise was greater with increasing acidity to the point where the change was over one pH unit in the most acid
Table 35. Preliminary Experiment to Determine Effect of Whey pH on NSI

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH of whey</th>
<th>pH of whey + defatted flour</th>
<th>NSI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>6.13</td>
<td>6.56</td>
<td>33.6</td>
</tr>
<tr>
<td>1</td>
<td>5.89</td>
<td>6.44</td>
<td>33.9</td>
</tr>
<tr>
<td>2</td>
<td>5.67</td>
<td>6.40</td>
<td>34.2</td>
</tr>
<tr>
<td>3</td>
<td>5.43</td>
<td>6.30</td>
<td>32.4</td>
</tr>
<tr>
<td>4</td>
<td>5.13</td>
<td>6.10</td>
<td>32.4</td>
</tr>
</tbody>
</table>

*No lactic acid added
<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Solids of wheys</th>
<th>pH of wheys</th>
<th>Titratable Acidity of wheys</th>
<th>Calcium Content of Wheys mg/100 ml</th>
<th>Total Solids of Wheys + Defatted Soy Flour %</th>
<th>pH of Wheys + Defatted Soy Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.58</td>
<td>6.31</td>
<td>0.072</td>
<td>37.5</td>
<td>11.51</td>
<td>6.57</td>
</tr>
<tr>
<td>1</td>
<td>6.54</td>
<td>5.83</td>
<td>0.111</td>
<td>37.4</td>
<td>11.49</td>
<td>6.36</td>
</tr>
<tr>
<td>2</td>
<td>6.55</td>
<td>5.23</td>
<td>0.160</td>
<td>37.4</td>
<td>11.32</td>
<td>6.12</td>
</tr>
<tr>
<td>3</td>
<td>6.53</td>
<td>4.69</td>
<td>0.238</td>
<td>37.5</td>
<td>11.24</td>
<td>5.71</td>
</tr>
</tbody>
</table>
Table 37 lists the NSI's of the mixtures before and after homogenization; effects of both single and double stage homogenization were evaluated. The results show a sharp decline in NSI because of acid development in the whey which was not overcome by homogenization. The bulk of the NSI reduction after homogenization occurred between the control and the sample prepared with whey of pH 5.83; values of these samples averaged only about 80% of controls. As acidity of the whey continued to develop, NSI values declined still further to the point where values were only 64 to 70% of controls. Although NSI increased as a result of homogenization, increases were greatest in the controls; homogenization pressure, whether single or double stage, had no effect in any of the samples prepared with the acid wheys.

Previous research (164) has shown that in order to prepare WSDM of good flavor quality and storage stability, the fluid sweet whey used for product manufacture should have a titratable acidity of 0.15% or less and a minimum pH of 5.9. From these results it is apparent that a lowering in pH of the whey used of only 0.5 unit can completely alter the solubility characteristics of the protein as measured by NSI, even though flavor quality is unimpaired. These results agree with those reported by Chen (105) who demonstrated that a slight alteration in pH and temperature alters the NSI value considerably in some cases.

Flow curves generated from Brookfield viscosity measurements are shown in Figures 58-64. In all cases, in contrast to the NSI
Table 37. Effect of pH of Whey on NSI of Defatted Soy Flour After Single and Double Stage Homogenization

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>pH 6.31 Control</th>
<th>pH 5.83</th>
<th>pH 5.23</th>
<th>pH 4.69</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NSI %</td>
<td>NSI %</td>
<td>NSI %</td>
</tr>
<tr>
<td>126.6</td>
<td>38.6</td>
<td>33.7</td>
<td>31.3</td>
<td>29.6</td>
</tr>
<tr>
<td>175.8</td>
<td>44.9</td>
<td>36.0</td>
<td>32.4</td>
<td>32.2</td>
</tr>
<tr>
<td>211.0</td>
<td>48.1</td>
<td>36.4</td>
<td>33.0</td>
<td>30.9</td>
</tr>
<tr>
<td>126.6-35.2</td>
<td>46.1</td>
<td>36.7</td>
<td>32.3</td>
<td>31.6</td>
</tr>
<tr>
<td>175.8-35.2</td>
<td>46.0</td>
<td>36.8</td>
<td>33.1</td>
<td>32.4</td>
</tr>
<tr>
<td>211.0-35.2</td>
<td>46.5</td>
<td>36.8</td>
<td>33.5</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>47.3</td>
<td>36.8</td>
<td>32.9</td>
<td>31.2</td>
</tr>
</tbody>
</table>
A = pH 6.57  B = pH 6.34  C = pH 6.09  D = pH 5.70

Viscosity flow curves of unhomogenized control samples of soybean flour in whey.

Figure 58
A = pH 6.57  B = pH 6.34  C = pH 6.09  D = pH 5.70

**Figure 59**

Viscosity flow curves of soybean flour in whey homogenized single stage at 126.6 kg/cm².
A = pH 6.57  B = pH 6.34  C = pH 6.09  D = pH 5.70

Viscosity flow curves of soybean flour in whey homogenized single stage at 175.8 kg/cm².

Figure 60
A = pH 6.57  B = pH 6.34  C = pH 6.09  D = pH 5.70

Viscosity flow curves of soybean flour in whey homogenized single stage at 211.0 kg/cm².

Figure 61
$A = \text{pH} \ 6.57 \quad B = \text{pH} \ 6.34 \quad C = \text{pH} \ 6.09 \quad D = \text{pH} \ 5.70$

Viscosity flow curves of soybean flour in whey homogenized double stage at 126.6-35.2 kg/cm$^2$.

Figure 62
Viscosity flow curves of soybean flour in whey homogenized double stage at 175.8-35.2 kg/cm².

Figure 63
A = pH 6.57  B = pH 6.34  C = pH 6.09,  D = pH 5.70.

Figure 64

Viscosity flow curves of soybean flour in whey homogenized double stage at 211.0-35.2 kg/cm².
values, there was little difference in viscosity among the controls and samples prepared with wheys of pH 5.83 and 5.23; however, flow curves for the samples prepared with whey of pH 4.69 were much lower than the others. Viscosities of all the samples increased with increasing homogenization pressure, although addition of the second stage (35.2 kg/cm²) resulted in only slight changes. Alteration of whey pH did not alter the shear stress-shear rate relationship in that flow was non-Newtonian and apparently pseudoplastic in nature.

A brief experiment was conducted to examine the effects of alkaline pH on NSI and viscosity of soybean flour proteins in both water and whey. Defatted soybean flour was saturated with ammonia gas and then dispersed in water or whey and homogenized at 211.0-35.2 kg/cm². The results are listed in Table 38 compared to controls prepared with flour that had not been ammoniated.

Treatment of the soybean protein with ammonia gas raised the pH from 6.7 to 9.2 when the flour was dispersed in water. Water plus dilute alkali in the pH range of 7 to 9 has been reported to increase the soybean protein extraction 5 to 10% (96); in this case, NSI was increased from 16 to 39% by ammonia treatment. Upon double stage homogenization at 211.0-35.2 kg/cm², NSI increased to 51% compared to 50% for the control.

The buffering action of the whey salt system reduced the pH to 8.15 when the ammoniated soybean flour was dispersed in whey; consequently, NSI was only increased by 4% over that of the control. Upon homogenization, NSI increased to 59% from 36.5% compared to an increase for the control to 43.6% from 32.7%. These results show that
Table 38. Effect of Saturation with Ammonia Gas on NSI of Soybean Flour Dispersed in Water or Whey Before and After Homogenization

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Solids %</th>
<th>No Homogenization</th>
<th>Homogenized 211.0-35.2 kg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control : water</td>
<td>5.37</td>
<td>16.3</td>
<td>49.5</td>
</tr>
<tr>
<td>pH 6.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₃ : water</td>
<td>5.38</td>
<td>39.1</td>
<td>80.9</td>
</tr>
<tr>
<td>pH 9.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control : whey</td>
<td>11.39</td>
<td>32.7</td>
<td>43.6</td>
</tr>
<tr>
<td>pH 6.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₃ : whey</td>
<td>11.06</td>
<td>36.5</td>
<td>59.3</td>
</tr>
<tr>
<td>pH 8.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
even in whey, the NSI increases if the pH is increased; the basic pH also improves the resolubilization of the proteins as a result of homogenization.

Flow curves from the Brookfield viscosity measurements are shown in Figure 65. In contrast to flow curves for viscosities measured in water or whey, at pH's of about 6.6 (Figures 52 and 56), after homogenization, the viscosities of the ammonia treated samples dispersed in water were greater than viscosities of the samples dispersed in whey, even though the total solids content was doubled. This effect is probably due to the more alkaline pH of the water dispersions; it is known that solutions of soybean globulins increase in apparent viscosity with increasing pH (74).

3. Effects of calcium ion on NSI

The effect of added calcium ion on the NSI of defatted soybean flour in water was also evaluated; the flour used in this experiment was a moderately toasted one. The results are shown in Table 39. NSI showed a sharp decrease when calcium ion concentration was increased from 50 to 200 mg/l and was reduced to 11.5% when the concentration was increased to 400 mg/l. This concentration is very close to the calcium concentration found in the whey used in the fermentation experiment (375 mg/l, Table 36). The NSI value found is only slightly higher than the NSI value for a fully toasted flour (NSI 18.7% in water) dispersed in whey (8.9%).

4. Effects of disulfide bond reducing agents on NSI and viscosity

The effects of treatment with disulfide bond reducing agents before and after homogenization at 211.0–35.2 kg/cm\(^2\) and 43.3°C were
A = Whey Control  B = Whey Homogenized  C = Water Control  D = Water Homogenized

Figure 65

Viscosity flow curves of ammonia gas-treated soybean flour in water or whey homogenized double stage at 211.0-35.2 kg/cm².
Table 39. Effect of Calcium Ion on NSI of Defatted Soy Flour in Water

<table>
<thead>
<tr>
<th>Sample</th>
<th>NSI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.6</td>
</tr>
<tr>
<td>No Added Ca++</td>
<td></td>
</tr>
<tr>
<td>50 mg Ca++/l</td>
<td>40.4</td>
</tr>
<tr>
<td>200 mg Ca++/l</td>
<td>22.8</td>
</tr>
<tr>
<td>400 mg Ca++/l</td>
<td>11.5</td>
</tr>
</tbody>
</table>
evaluated for their effects on the NSI and viscosity of defatted soy flour dispersed in water or whey. The results for samples treated with the reducing agents before homogenization are shown in Table 40.

Treatment with both 2-mercaptoethanol and sodium bisulfite increased the NSI of the soy flour proteins over that of the control in both water and whey without homogenization. However, homogenization in the presence of the reducing agents resulted in great increases in NSI values over values of the untreated controls with the greatest increase being found in the whey samples.

Table 41 lists the NSI values found when aliquots of the homogenized controls, both water and whey, were treated with the disulfide bond reducing agents. Both reducing agents increased NSI of the flour samples in water but the increases observed were only one-half of the increases brought about by homogenization in the presence of the two additives. Increases in NSI were even less when the samples homogenized in whey were treated with the two reducing agents.

Flow curves from the Brookfield viscosity measurements for the samples, un-homogenized or homogenized in water or whey, are shown in Figures 66, 67 and 68. Apparent viscosity values were very low for the un-homogenized samples dispersed in water (Figure 66) and differed very little from one another. Upon homogenization at 211.0-35.2 kg/cm², both samples treated with reducing agents showed pseudoplastic flow with yield stress whereas the control did not. This could be experimental error because great difficulty was encountered in measuring viscosities on these samples because of the rapid settling. The un-homogenized samples dispersed in whey had viscosities slightly higher
Table 40. Effects of Homogenization on NSI of Defatted Soy Flour Dispersed in Water or Whey and Treated With Disulfide Bond Reducing Agents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Solids %</th>
<th>No Homogenization</th>
<th>211.0-35.2 kg/cm²</th>
<th>Total Solids %</th>
<th>No Homogenization</th>
<th>211.0-35.2 kg/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.41</td>
<td>17.5</td>
<td>53.0</td>
<td>11.39</td>
<td>32.7</td>
<td>43.6</td>
</tr>
<tr>
<td>No Treatment</td>
<td>10 mMol/l 2-mercaptoethanol</td>
<td>5.32</td>
<td>37.5</td>
<td>72.2</td>
<td>11.43</td>
<td>44.6</td>
</tr>
<tr>
<td></td>
<td>7.5 mMol/l sodium bisulfite</td>
<td>5.45</td>
<td>31.9</td>
<td>70.0</td>
<td>11.28</td>
<td>43.0</td>
</tr>
</tbody>
</table>
Table 41. Effects of Disulfide Bond Reducing Agents on NSI of Defatted Soy Flour Dispersed in Water or Whey After Homogenization at 211.0-35.2 kg/cm²

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenized Control</td>
<td>53.0</td>
<td>43.6</td>
</tr>
<tr>
<td>Homogenized Control + 10 mMol/l 2-mercaptoethanol</td>
<td>60.9</td>
<td>53.4</td>
</tr>
<tr>
<td>Homogenized Control + 7.5 mMol/l sodium bisulfite</td>
<td>59.3</td>
<td>52.6</td>
</tr>
</tbody>
</table>
Shear Rate

Viscosity flow curves for control samples of soybean flour homogenized double stage at 211.0-35.2 kg/cm² in water or whey.

Figure 66
A = Water  B = Water Homogenized  C = Whey  D = Whey Homogenized

Viscosity flow curves for 2-mercaptoethanol-treated samples of soybean flour homogenized double stage at 211.0-35.2 kg/cm² in water or whey.

Figure 67
Viscosity flow curves for sodium bisulfite-treated samples of soybean flour homogenized double stage at 211.0-35.2 kg/cm² in water or whey.

Figure 68
than the water samples; flow appeared to be very close to Newtonian and the curves fell on top of one another. Upon comparison of the curves for the homogenized samples, it is evident that the presence of the additives inhibited viscosity build-up even though there was a large increase in NSI in these samples (Table 40).

5. Effects of 2-mercaptoethanol on phase separation

Effects of 2-mercaptoethanol on phase separation after reconstitution of WSDM powder to 15% total solids and 24 hours of standing in the cold were evaluated. Powder samples used were the 6 experimental powders prepared to test the RSM computer predictions because they were the only freshly made samples available. In addition, the commercially manufactured Sample C was tested even though the sample was several years old.

The results, listed in Table 42, show that 2-mercaptoethanol had little effect on the phase separation of the samples of Set I, homogenized at 175.8-35.2 kg/cm² and about 14% total solids. However, phase separation was increased sharply by 2-mercaptoethanol treatment of the Set II samples, homogenized at 211.0-35.2 kg/cm² and about 20% total solids. The samples of Set III also showed slightly increased phase separation. Phase separation of the commercial sample was also increased considerably by the treatment.

The supernatant of sample C was analyzed for total solids, total protein and total fat to determine if 2-mercaptoethanol treatment altered the composition; the results are shown in Table 43. Even though the sample was old, the analysis of the untreated control agreed closely with the original compositional analysis of the
Table 42. Effect of 2-Mercaptoethanol on Phase Separation After 24 Hours Refrigerated Storage (15\% TS)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>2-mercaptoethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mMol/l</td>
<td></td>
</tr>
<tr>
<td>Set I, No. 1</td>
<td>69.1</td>
<td>73.0</td>
</tr>
<tr>
<td>Set I, No. 2</td>
<td>69.8</td>
<td>75.0</td>
</tr>
<tr>
<td>Set II, No. 1</td>
<td>17.6</td>
<td>63.9</td>
</tr>
<tr>
<td>Set II, No. 2</td>
<td>27.4</td>
<td>70.8</td>
</tr>
<tr>
<td>Set III, No. 1</td>
<td>19.2</td>
<td>27.4</td>
</tr>
<tr>
<td>Set III, No. 2</td>
<td>17.0</td>
<td>24.2</td>
</tr>
<tr>
<td>Commercial Sample C</td>
<td>23.3</td>
<td>43.6</td>
</tr>
</tbody>
</table>
Table 43. Effect of 2-Mercaptoethanol on Composition of Wheyed-Off Supernatant of a Commercially Prepared WSDM (Sample C)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2-mercaptoethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS/15% TS x 100</td>
<td>61.4</td>
<td>71.2</td>
</tr>
<tr>
<td>Protein/Total Protein x 100</td>
<td>29.5</td>
<td>46.8</td>
</tr>
<tr>
<td>Fat/Total Fat x 100</td>
<td>1.20</td>
<td>37.1</td>
</tr>
</tbody>
</table>
wheyed-off supernatant (Table 15). The results showed that 2-mercaptoethanol treatment increased both the protein and fat contents of the supernatant; the increase in fat was particularly striking because of the very low fat content found in the control supernatant. These results suggested that the breaking of the accessible disulfide linkages by 2-mercaptoethanol altered the charge on the dispersed soybean proteins so that less fat was bound; therefore, the particle size diameter may have been altered.

The average equivalent spherical diameter de was measured on defatted soybean flour dispersed in water and on the reconstituted samples of Sets I, II and III; results are shown in Table 44. The results were variable but appeared to show little discernable differences in de as a result of 2-mercaptoethanol treatment.

6. Ultracentrifuge studies

Ultracentrifuge analysis was used to determine what effect homogenization had on the major soybean proteins found in the defatted flour. The ultracentrifuge patterns, before and after homogenization at 211.0-35.2 kg/cm² for defatted flour slurried in water are shown in Plate XII. The patterns appear to be the characteristic patterns for soybean globulins reported by Nash and Wolf (77).

Table 45 shows the Svedberg coefficients and peak area percentages contributed by each peak. Homogenization appears to have reduced the concentration of the 2S protein while increasing that of the 11S protein. Areas of the other two peaks increased only slightly.
Table 44. Effect of Treatment with 2-Mercaptoethanol on de

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>10 mMol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>de &gt;8 micrometers</td>
<td>Cumulative weight per cent</td>
</tr>
<tr>
<td>Defatted flour</td>
<td>14.76</td>
<td>89.3</td>
</tr>
<tr>
<td>Set I, No. 1</td>
<td>12.75</td>
<td>84.3</td>
</tr>
<tr>
<td>Set I, No. 2</td>
<td>12.08</td>
<td>78.3</td>
</tr>
<tr>
<td>Set II, No. 1</td>
<td>12.66</td>
<td>84.3</td>
</tr>
<tr>
<td>Set II, No. 2</td>
<td>12.35</td>
<td>82.1</td>
</tr>
<tr>
<td>Set III, No. 1</td>
<td>12.90</td>
<td>85.8</td>
</tr>
<tr>
<td>Set III, No. 2</td>
<td>13.12</td>
<td>86.3</td>
</tr>
</tbody>
</table>
Plate XII. Ultracentrifuge schlieren patterns of soybean proteins.

A = Control
B = Homogenized 211.0-35.2 kg/cm²
Table 45. Effect of Double Stage Homogenization at 211.0-35.2 kg/cm² on Svedberg Coefficient and Peak Area of Ultracentrifuge Patterns of Proteins From Toasted Defatted Soybean Flour Protein

<table>
<thead>
<tr>
<th>Peak</th>
<th>Svedberg Coefficient</th>
<th>Per Cent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Homogenized</td>
</tr>
<tr>
<td>1</td>
<td>1.58</td>
<td>1.90</td>
</tr>
<tr>
<td>2</td>
<td>7.36</td>
<td>7.30</td>
</tr>
<tr>
<td>3</td>
<td>11.74</td>
<td>11.21</td>
</tr>
<tr>
<td>4</td>
<td>17.00</td>
<td>16.42</td>
</tr>
</tbody>
</table>
Although the patterns show only 4 peaks, the Svedberg coefficients for the fast moving peak, before and after homogenization, are both greater than 15S; a 15S peak is absent.

These results suggest that the soybean proteins, especially the 11S globulin, that are insolubilized by the heat treatment received during flour manufacture are partially resolubilized by homogenization.
DISCUSSION

This study was undertaken to explore the effects of some processing parameters, known to affect the powder structure and reconstitutability of milk powders, on the physical characteristics of a spray dried milk analogue containing soy products. Processing effects on the physical stability of the reconstituted beverage were of particular interest.

Under the experimental conditions, emulsifier had an adverse effect on the rehydration properties examined. However, homogenization brought about a significant increase in NSI which was maintained through the drying stage. Average particle size of the dispersed particles was not the sole factor responsible for the lack of physical stability upon reconstitution. Homogenization was beneficial in that a fat-protein complex was formed that contributed to improved suspension stability after reconstitution; however, pressures used were not sufficient to eliminate phase separation completely.

A. Structural Studies

Microscope studies have shown that intact spray dried WSDM powder particles resemble those of spray dried nonfat milk powder. The major difference from NDM is in the appearance of the fractured surfaces (Plate V). The pitted fracture surface of WSDM might be caused by the dispersion of the lipid in the aqueous phase before drying. This pitting has been reported by Buma and Henstra (368) as
being typical of broken particles of spray dried whole milk powder observed by SEM; no such pitting was observed by these authors in spray dried NDM, whey or sodium caseinate. The authors attributed the pits to holes that had been previously occupied by the dispersed fat droplets; however, nothing in the preparation of their samples for SEM microscopy or of the WSDM sample preparation should have extracted the lipid. Therefore, it is concluded that when the lipid is well dispersed, as by homogenization, the spatial arrangement of the other more soluble components in the drying atomized droplet is disrupted by the oil in water emulsion during the process of water evaporation resulting in the pitted appearance in the dried particle. These results show therefore that spray dried WSDM bears a closer resemblance to whole milk powders than to the nonfat dry milk that it is intended to replace.

Microscopy has also shown that, at least during the initial stages of reconstitution, the dissolving WSDM particle begins to break up and disperse into the medium surrounding the particle in a manner similar to that of whole milk powder but not as rapidly. This may be accounted for by assuming that the soy components contributed by the defatted flour used in powder manufacture do not wet as rapidly as do the milk components. The presence of a roughened but almost intact powder particle after reconstitution (Plate VIII) backs up this assumption; nonfat dry milk, reconstituted and examined by SEM under similar conditions showed no such particles (Plate not included).

After reconstitution and standing, characteristics generally attributed to soy milks became obvious. Many of the SEM
photomicrographs taken after WSDM was reconstituted showed particle characteristics similar to those observed by Wolf and Baker (18) in SEM studies of soy flours, concentrates and isolates. The size of this particulate matter could be measured by Coulter counter.

Further studies with differential staining of the components of the reconstituted WSDM clearly showed the association of fat droplets with the particulate material. This association is undesirable as far as initial wetting and dispersion characteristics are concerned because the hydrophobic surface presented to the dispersing aqueous medium would inhibit aggregate dispersal. However, the association may ultimately be a desirable feature for reducing the amount of phase separation on standing after reconstitution because creaming would be reduced. In addition, the density of the dispersed particles would be altered because the associated fat could make the settling particles more buoyant.

1. Powder particle size distributions

Results for the powder particle size distributions obtained by microscopy were disappointing but could be accounted for by two factors: 1. Measurement of an insufficient number of particles and 2. Variation in total solids of the concentrates fed to the spray dryer. In spite of these difficulties, particle size distributions of the powders were analyzed and found to exhibit characteristics similar to those for some milk powders (201).

The shapes of the curves obtained for the experimental and commercially prepared WSDM powders showed that the distributions did not follow a log normal distribution function. Modified log
normal distributions are frequently found in powders prepared by spray
drying or by fractionation such as that used for flour preparation.

There are several mathematical interpretations available
that are useful for describing powders obtained by controlled forma-
tion, spray drying or fractionation. These treatments can also be
applied to the particle size distribution obtained by Coulter Counter.
Irani and Callis (196) have treated these in some detail and the
following discussion is taken from their book.

The size $M$ of a particle can grow or diminish according to

$$\frac{dM}{dt} = \Phi (M)$$

where $\Phi (M)$ is expressed as

$$\Phi (M) = K \frac{(M - M_0)(M_\infty - M)}{(M_\infty - M_0)}$$

$M_\infty$ and $M_0$ are the maximum and minimum particle sizes formed and $K$ is
a velocity constant of formation, either positive or negative depend-
ing on whether the particle is growing or being destroyed. As $M$
approaches either $M_0$ or $M_\infty$, it becomes time dependent because $\Phi (M)$
becomes zero. Therefore, the time distribution would be

$$f(t) = \frac{1}{\sqrt{2\pi}} \exp \left\{ \frac{-t^2}{2} \right\}$$

assuming a unit standard deviation, because time units can be in-
creased or decreased at will; the assumption can be made that times
of growth or destruction of particles are normally distributed.

If equations (3) and (4) are combined, then

$$t = a + b \ln \left( \frac{(M - M_0)(M_\infty - M_0)}{(M_\infty - M)} \right)$$
where a and b are constants. Then if equations (5) and (6) are combined

\[ f(M) = \frac{1}{\sqrt{2\pi} \ln\sigma} \exp \left\{ -\left[ \ln \left( \frac{M-M_0}{M_\infty-M_0} \right) \right]^2 \right\} \]

where \( \sigma \) and \( M_\bar{\sigma} \) may be temporarily assumed to be constants related to a and b. In the case where \( M_0 \approx 0 \), and \( M_\infty \) is very large, equation (7) reduces to

\[ f(M) = \frac{1}{\sqrt{2\pi} \ln\sigma} \exp \left\{ -\left[ \ln \left( \frac{M}{\bar{M}} \right) \right]^2 \right\} \]

the simple log-normal distribution law.

Equation (7) can be integrated to yield \( P \), the percent greater than size \( M \).

\[ P = 100 \int_{M}^{\infty} f(M) d\ln \frac{M-M_0}{M_\infty-M_0} \]

\[ = 50 - 100 \text{erf} \left[ \ln \frac{(M-M_0)(M_\infty-M_0)}{\bar{M}(M_\infty-M_0)} \right] \]

where \( \text{erf} \) = the error integral and \( \bar{M} \) is the geometric mean size of the parent distribution.

If \( M_0 = 0 \) and \( M_\infty \to \infty \), equation (10) reduces to the simple log normal distribution and yields a straight line on the log probability axis. This case corresponds to unlimited growth of the
particle.

Other cases involve modifications in the log-normal distribution due to variables controlling powder particle formation.

In the first of these, $M_\infty \rightarrow 0$; however, $M_0 \neq 0$ but $M_0 > 0$. This means that on log probability paper, a curve will be obtained that is asymptotic toward the lower size limit. If $(M - M_0)$ is plotted rather than $M$, a straight line can be obtained.

If $M_0 = 0$ but $M_\infty \neq \infty$, a curve is obtained that is asymptotic toward the upper size limit. The majority of the log probability plots for the experimental WSDM powders fall into this category; an example is the curve shown in Figure 69 representing data for the sample homogenized at 26% total solids at 211.0-35.2 kg/cm$^2$ without emulsifier (Figure 21). The curve for commercial sample A (Figure 10) also appears to fit in this category.

If $M_0 / (M_\infty - M)$ rather than $M$ is plotted on log probability paper, a straight line should be obtained. Assuming an upper size limit value of 94 micrometers for $M_\infty$, it can be seen from the replotted line shown in Figure 69 that a straight line is obtained. This modification is frequently found with spray dried powders; it is usually due to variables controlling the formation of the dried particle such as the spray pressure and orifice size of the atomizer on the spray dryer.

Modifications in the log normal distribution may also be brought about by artificially altering the size distribution after the particles are formed. Such changes can occur through dust separation by cyclones or by screening or crushing of the powder.
Figure 69: Log probability plots of particle size distributions of experimental WSDM powder homogenized at 211.0-35.2 kg/cm² and 26% total solids without emulsifier.
after drying.

There was no deliberate alteration in powder particle
size of the experimental WSDM powders, because the dryer used was not
equipped with a cyclone, nor was the powder screened or crushed after
collection.

Multimodal particle size distributions may also occur and
arise from mixing powders, or from the existence of different rates
and boundary conditions during particle formation.

Two types of distributions may occur if it is assumed
that \( M_0 = 0 \) and \( M^\infty \rightarrow \infty \). In the first type, the parent log normal
distribution curves do not intersect on a log probability plot; the
curve asymptotes at the upper and lower ends to two nonhorizontal
lines, that are the parent distributions.

Equation (10) becomes

\[
P = 50 - 100 f \text{ erf} \left[ \frac{\ln M/M_1}{\ln \sigma_1} \right] - 100 (1-f) \text{ erf} \left[ \frac{\ln M/M_2}{\ln \sigma_2} \right] \quad \ldots \quad (11)
\]

where \( f \) is the fraction \( f_1 \). An approximation of \( \sigma_1 \) and \( \sigma_2 \) can be
obtained from the asymptotes of the experimental points and \( f \) can
then be computed at various \( P \) values. Estimates of \( M_1 \) and \( M_2 \) can be
made from the size frequency histogram.

In another case of multimodal distribution, the parent
log normal distribution curves intersect on a log probability plot.
The asymptotes of the curve do not approach specific size values but
asymptote at both ends of the distribution toward the parent distribu-
tion with the higher \( \sigma \). The inflection point is very important
because both parent curves pass through it. $\bar{M}_1$ and $\bar{M}_2$ are obtained from a size frequency histogram; the inflection point is determined by drawing tangents to the curve and finding the point of maximum change in slope. $\bar{M}_1$ and $\bar{M}_2$ are located at 50% probability and connected to the inflection point by straight lines to obtain $\sigma_1$ and $\sigma_2$. The $f$ values can then be computed from equation 9 at various measured values of $P$ and $M$.

The shapes of the curves for commercial samples B, C and D (Figures 11, 12 and 13) suggest a bimodal size distribution of the second type; some of the experimental WSDM powders also appeared to have this type of distribution (Figure 22). The bimodality in the distribution was probably caused by powder aggregation in the dryer after atomization or by agglomeration after drying, resulting in a few very large particles. However, this is not clearcut because of the limited number of particles measured in the upper size ranges so experimental error could be responsible for curve shape for the experimental WSDM powders. The commercial powders contained many aggregates, especially sample D; in addition, the commercial dryers were undoubtedly equipped with dust collectors so some alteration in particle distribution could easily have occurred.

The use of full fat or defatted flour did not appear to have an effect on the average particle diameters of the commercially prepared WSDM powders (Table 16). All diameters were considerably larger than those for the experimental WSDM's however, probably reflecting the difference in the drying parameters.
3. Physical Measurements

The physical measurements of dispersibility, sinkability, ADM solubility index and free fat content have been in common use as a means of describing the reconstitution characteristics of whole and skimmed milk powders (210). The results reported here show that these measurements in slightly modified form in some cases, have supplied an adequate description of the initial rehydration characteristics of a model spray dried milk analogue, WSDM, so that different powders might be compared. By projection, these measurements might also be used to characterize the reconstitutability of other dehydrated milk analogues. Because these tests are familiar to the dairy industry, official procedures might be developed for evaluating such analogues after the present tests have been refined somewhat and some new tests developed.

This study has shown that many of the initial rehydration characteristics of WSDM resemble those of the milk powder it is intended to replace; after reconstitution, however, properties differing from those of reconstituted milk powders but resembling those of soybean beverages, become apparent. Therefore, three additional parameters, the NSI, the average equivalent spherical diameter de, and the degree of phase separation after $24$ hours of standing in the cold after reconstitution were selected for evaluation for their ability to describe the rehydrated system further.

It was evident at the beginning that measurement of phase separation after only one hour of standing after reconstitution was inadequate probably because wetting of all components in the whey-soy
system was not completed in that time. This test is required by specifications for WSDM (159); based on results reported here, this test should either be eliminated or replaced by a test for phase separation after 24 hours of rehydration.

The NSI and the ADMI solubility index are both measures of solubility. Because of the large quantity of insoluble material in the WSDM system, it was necessary to modify the ADMI test somewhat from that normally used for measuring the solubility index of milk powders. Results from the modified test, crude though it is, were found to parallel those obtained by the standard NSI test for cereal products. Therefore, the modified ADMI solubility index test may be used as a measure of solubility of WSDM especially when comparing a large number of powders to one another in a limited time period. For more accurate results, the NSI test should be used, even though a much longer time is required to complete the analysis.

Dispersibility measurements also provided an adequate means for comparing one WSDM powder to another. A direct comparison of dispersibility values to those reported for whole and skim milk powders should not be attempted because it was necessary to modify the test used. The large amount of dispersed but insoluble material contributed by the soybean flour clogged the 40-60 micrometer filter used when evaluating dispersibilities of milk powders (211) so this filter was eliminated.

Sinkability measurements were not as satisfactory but could also be used to compare one powder to another provided moisture contents were similar and emulsifier was absent. Sinkabilities of WSDM
prepared without emulsifier were shown to have a linear dependency on moisture content in that powders of higher moisture sank faster. This means that the particles were denser and consequently were able to penetrate the air-water interface more rapidly.

Measurements of free fat also proved to be a helpful means of comparison for WSDM powders. Contents measured were found to be similar to those reported for spray dried whole milk powders (226) so it was concluded that this test could be used without modification to provide some information about the physical state of the lipid phase in spray dried powders containing lipids other than milk fat.

The particle size distribution has been reported to be an important factor in the suspension stability of soybean beverages (173). In the traditional method for Chinese soymilk manufacture, stability is improved because the slurry is filtered after grinding (116). In a Coulter Counter study with the "Illinois soybean beverage," Nelson et al (146) reported that there were no particles below 2.7 micrometers in diameter and about 10% of the particles were larger than 10 micrometers. Therefore, it was anticipated that measurement of the average equivalent spherical diameter, de, could provide information which could be related to the suspension stability of the reconstituted powders.

Unfortunately, it was not possible to compare particle size distributions of dispersed particles of WSDM to other values reported in the literature for soy products. The size distributions of the dispersed particles were altered by artificially excluding all particles below 8 micrometers in diameter from the count. However, the
distributions shown in Figure 23 suggest that the average particle diameters of the reconstituted WSDM powders would still have been larger than those reported by Nelson et al even if it had been possible to include the smaller particles in the counts.

The results showed that de could not be clearly related to phase separation after only one hour of standing; after 24 hours, however, de did prove to be related to phase separation. It was concluded from this therefore that de was an appropriate measurement when considering rehydration properties of milk analogues containing soy products. It was also concluded, in agreement with Nelson et al (146), that particle size alone was not the controlling factor in suspension stability.

C. Processing Variable Effects

1. Dispersibility

The literature survey showed that processing steps preceding spray drying of milk powders generally had little effect on dispersibility. Self dispersion properties were reduced by high preheat temperatures and by homogenization before or after condensing (227) but these effects were minor compared to those of the drying parameters. Drying parameters are important because of their effects on particle structure and particle size distribution.

Therefore, even though RSM analysis predicted a high correlation (.98) of dispersibility of WSDM with the processing of homogenization pressure and total solids of homogenization, the lack of agreement of the observed results with the predicted values (Table 23) led to the conclusion that drying parameters must also be
the major influence on the dispersibility of WSDM.

It has been reported that, within limits, the rate-of-dissolution of small powder particles is greater than that of large ones because the rate of dissolution of particulate matter is dependent on the specific surface in contact with the solvent (370, 371). However, in the case of milk powders, large particles, more irregular in shape, result in better wettability and more rapid dissolution (214, 266); on the other hand, if bulk density is too great, self dispersion is decreased (265).

It had been expected that the dispersibility of the experimental WSDM powders would show a linear correlation with the powder particle diameter related to the specific surface area of the powder (\(\bar{d}_3\)). This was not the case but can probably be accounted for by unavoidable variation in the drying parameters during powder manufacture and experimental error brought about by measurement of insufficient powder particles for particle size distribution calculations.

Dispersibility values were significantly correlated with \(d_e\). This was difficult to explain. However, SEM studies showed that WSDM powder particles were slow to dissolve. Dispersed but undissolved particles persisted in the medium after reconstitution; this could account for the relationship between dispersibility and \(d_e\).

The decrease in dispersibility of WSDM in the presence of emulsifier is in apparent contrast to results reported by Mather and Hollender (262) for milk powders containing hydrophilic surface active agents. These authors reported a significant increase in
self-dispersion properties but the more hydrophilic surface active agents promoted fat churning during mechanical reconstitution if the concentration exceeded 0.06%.

Improved sinkability is considered to be an improvement in self-dispersion (260). These authors also reported fat churning with the use of higher concentrations of surfactants but sinkability was enhanced. Results of WSDM with emulsifier agreed with those of Nelson and Winder in that sinkability (wettability) was enhanced. However, in no case was fat churning observed in WSDM powders during the tests where vigorous agitation was required (NSI, ADMI solubility index). Soybean oil is a liquid at room temperature; low melting lipids have a lower interfacial tension toward water than higher melting lipids such as milk fat. Therefore, the combination of the two factors, physical state of the lipid and the interfacial tension, probably prevented churning when WSDM was reconstituted.

The decrease in dispersibility in the WSDM samples with emulsifier was undoubtedly due to the increase in free fat. Unprotected free fat will coalesce and form patches on the surface of the powder particles, making them water repellent (210) and less dispersible. The dispersibility decrease could also be related to the slight decrease in soluble nitrogen brought about by the emulsifier. Some of the WSDM components are apparently slow to wet initially and anything that slows this process further could reduce dispersibility.

2. Free fat

The free fat content is considered by many to be one of the most important quality attributes of dry whole milk (372). A high
free fat content is undesirable from the standpoint of preservation, 
dispersibility and free-flow characteristics of the powder.

Verification of RSM predictions for minimizing free fat 
content of WSDM showed that although the trends were correctly pre-
dicted, free fat contents actually found were higher in all cases.
This means that even though the processing variables studied had some 
influence on free fat levels in the dry powder, other factors must 
also be involved.

Experimental WSDM samples, homogenized single or double 
stage with emulsifier at 29\% (single) or 26\% total solids (double) 
had higher free fat contents than similar samples homogenized at 12.6\% 
(single) or 13.7\% total solids (double). Only the emulsifier was 
shown to have a significant effect on the free fat content of samples 
homogenized single stage.

In the samples homogenized double stage, the total solids 
of the mixture homogenized also was found to be significant because 
the free fat content was much lower in the samples homogenized at 40\% 
total solids whether or not emulsifier was present. It must be 
pointed out that this sample set also received an additional homogeni-
ization (single stage at 84.4 kg/cm$^2$) to disperse the oil before con-
densing; therefore this could be partly responsible for the much 
lower free fat content observed.

Although it was not evident in the experiments with WSDM, 
the influence of homogenization pressure and total solids of the 
mixture homogenized on the free fat content of whole milk powders is 
important.
Homogenizing the concentrate before drying caused a noticeable decrease in free fat content of the powder (236, 373). Increasing the total solids of the concentrate fed to the dryer also decreased the free fat content of the powder regardless of whether the concentrate was homogenized (239). Free fat content was decreased further by homogenization and the decrease was greater with increased homogenization pressure and with increased total solids of the concentrate. On the other hand, increasing the concentrate temperature before drying increased the free fat content, the increase being greater with increased total solids of the concentrate.

Some of the variation in the free fat contents observed in the WSDM powders can therefore be explained by variation in the total solids of the concentrates fed to the dryer.

Homogenizing effects of the centrifugal atomizer used on the free fat content of the WSDM powder must also be considered. With whole milk concentrates, if the total solids content is low, the homogenizing effect is also low; this means that the viscosity and consequently the frictional forces in the liquid flow on passing through the atomizer are low. Concentrates with high total solids are much more viscous; they are subject to many internal frictional forces that clearly result in a finer dispersion of fat globules and a further reduction in free fat content (239, 374). The viscosities of the concentrates homogenized double stage at 40% total solids were considerably higher than concentrates homogenized at lower total solids levels. Therefore the increased homogenization effect occurring during drying of this sample set could also be a factor in the
low levels of free fat found even in the samples with emulsifier.

Buma (236) demonstrated that homogenization of a whole milk concentrate before drying decreased free fat content because homogenization decreased particle porosity of the dry powder. DeVilder et al (372) showed a linear relationship for whole milk powders between the free fat content and nitrogen gas penetration into the powder particle; this also proved to be true for the permeability factor $\gamma_{10}$ described by Buma (276). DeVilder et al (372) confirmed Buma's theory that the free fat does not comprise just surface fat but also all the fat in the cracks and capillaries accessible to the fat solvent.

DeVilder et al (372) pointed out that the decrease in free fat content between whole milk powders prepared from unhomogenized concentrates and those prepared from concentrates homogenized double stage could not be explained only by the differences in powder porosity. The free fat content of such powders dried from concentrates homogenized double stage was lower than would be expected from permeability and nitrogen penetration measurements. The authors believed that this difference is due to the formation of a new fat membrane during the homogenization process. Fox et al (375) have shown that a fat-protein complex is formed during homogenization of whole milk. The protein moiety associated with the complex is casein and the association is probably through apolar bonding forces. The proportion of casein in the complex increased with increased homogenization pressure. The casein micelle may have been activated as it passed through the homogenizer valve, predisposing it to interaction
with the lipid phase. Henstra and Schmidt (376) and Buchheim (377)
confirmed with electron microscope studies that a fat-protein complex
did form with homogenization. The fat globules became coated with a
layer of casein, an effect that increased with increased homogenization
pressure. In addition, at high homogenization pressures (350
atm) (361.66 kg/cm²) agglomerates were formed of fat globules,
cemented together with casein. Henstra and Schmidt (376) concluded
that the internal structure of the casein micelles was broken down
during homogenization, thus providing a possibility for the casein
subunits to bind to the fat globule surface. Iwaida and Tsugo (378)
also suggested that high pressure homogenization (> 400.8 kg/cm²)
altered the casein micelle. Tamsma et al (379) suggested that
homogenization could rupture casein micelles into more surface-active
particles. DeVilder et al (372) suggested that the altered surface
membrane brought about by the lipid-protein interaction might protect
the fat globules against extraction during free fat measurements.

Of the three processing variables studied, the addition
of emulsifier had the greatest effect on the free fat content of WSDM.
Structural studies showing fat droplets adsorbed to the particulate
surfaces coupled with the lack of fat present in the supernatants of
phase separated (by gravity) reconstituted WSDM powders formulated
without emulsifier suggests that homogenization is bringing about
some type of lipid-protein interaction that is reduced by the addition
of emulsifier, at least with the type and concentration used. That
the observed interaction is weak, perhaps hydrophobic in nature or
electrostatic attraction, is suggested by the increase in fat content
of the supernatants obtained during the NSI test; when mechanical energy is put into the system as with the vigorous stirring and centrifugation required during the NSI test, the lipid-protein bonding is broken, releasing the fat. Results reported by Fox et al (375) for complex formation in whole milk showed little difference in the amount of complex formed in the range of homogenization pressures used for the WSDM experiments until the total solids of the concentrate homogenized was increased above 31%. Above this total solids level, a significant increase in the amount of fat bound occurred, even at low homogenization pressures.

Results for WSDM suggest that the homogenization pressures used are not sufficient to produce a significant amount of lipid-protein complex even when the total solids of the mixture homogenized was raised to 40%. Some lipid-protein interaction was occurring because free fat was lowest in this sample set and the percentages of fat in the soluble nitrogen fractions were significantly affected by all three processing variables. Increasing the homogenization pressure during WSDM manufacture to levels above those normally used in industry could reduce the free fat content further by strengthening the lipid-protein interaction.

The purpose of an emulsifier in the system is two-fold: to lower the surface tension at the interface of two immiscible liquids so that droplets of one of them can form and to stabilize the droplets, preventing them from coalescing, aggregating and separating into layers.
Although many surface active agents can act as emulsifiers, no one agent can meet the needs of all emulsions. A surfactant that can act as an emulsifier in one formula can cause instability in others; this instability is usually observed as oil separation or churning. Although no churning was observed in fresh WSDM powders on reconstitution, the emulsifier, Tween 60, at the concentration that was used in the WSDM formulation, increased the free fat content of the resultant powders in all cases.

Mather and Hollender (262) found that Tween 81 (polyoxyethylene sorbitan monostearate), at a concentration of 0.1% of the fluid, promoted churning during reconstitution of whole milk powders, even though dispersibility was improved. By altering the hydrophilic-lipophilic balance by using a combination of two materials such as 0.05% Tween 60 (HLB 14.9) and 0.05% Span 62 (sorbitan monostearate, HLB 4.7), good self-dispersibility with no churning could be achieved. The more hydrophilic surfactants such as the Tweens promoted the churning effect.

Nelson and Winder (260) found that surfactants enhanced sinkability of whole milk powders but fat churning occurred on reconstitution if Tween 60, Tween 80 or lecithin were added to the whole milk or whole milk concentrate at concentrations greater than 1% by weight of the fat. They found that best sinkability with almost no churning was obtained with a mixture of Tween 80 (HLB 15) and Atmos 300 (HLB 2.8) yielding an HLB of 8.0.

Free fat contents of the milk powders were not reported in these two studies. However, because churning represents a
deemulsification of a type, it seems logical that the more hydrophilic surfactants increased the free fat content of the milk powders, just as Tween 60 did with WSDM. An area of future work could therefore be to determine if alteration in the emulsifier HLB and concentration could enhance WSDM dispersibility while improving homogenization efficiency.

The release of free fat in the WSDM powder is indicative of destabilization, probably during the drying process. In ice creams, surfactants function in two ways: before freezing, they keep the fat dispersed and in suspension in the mix whereas in the freezer, surfactants produce a drying effect by aiding controlled destabilization of the fat emulsion, promoting agglomeration of the fat globules (380). This drying action is entirely different from a surfactant application in causing oil and water to mix. Research (381, 382) has shown that dryness in ice cream is achieved by forcing the finely dispersed fat globules to agglomerate. Microscopic examination shows that the fat in ice cream mix is well dispersed in globules 1-2 micrometers in diameter. As the mixture is agitated and frozen, the surfactant induces the fat globules to cluster together like bunches of grapes; the amount of dryness brought about by the agglomeration is determined by the amount and type of emulsifier.

It is postulated that a similar effect is occurring in WSDM during the drying step. The removal of water, whether by freezing or by evaporation would alter the orientation of the surfactant molecule at the fat globule surface. The hydrophilic portions of the surfactant could orient toward one another with increasing solute
concentration, resulting in fat globule clustering and subsequent destabilization, thereby increasing the amount of extractable (free) fat.

3. NSI

The NSI of WSDM was found to be significantly affected by all three processing variables. The effects of heat on the NSI of soybean proteins have been described in detail (9). However, the finding that the reduced NSI of a fully toasted soybean flour could be reversed by a simple processing procedure such as homogenization and that this improvement would persist through a dehydration step is significant and has not been previously reported. This shows that any processing operation that tends to have a homogenizing or comminuting effect could increase NSI, thereby altering the functional characteristics; this could partially account for Chen's (105) report that NSI could not be used satisfactorily to predict certain functional characteristics of soy isolates.

Aminlari et al (174) were able to increase the PDI of their spray dried beverage base by treating the mixture with sodium bisulfite before drying. As sodium bisulfite is fairly specific for disulfide bonds, it seemed probable that depolymerization of the proteins occurred through cleavage of disulfide bonds. Although Aminlari et al observed an increase in PDI of the dried powder as homogenization pressure of the fluid mix increased to a maximum of 386.8 kg/cm², they attributed the PDI increase to a reduction in particle size. The decrease in PDI observed when pressures greater than 386.8 kg/cm² were used were attributed to adverse effects of pressure on the soybean
Results reported here show that the increased NSI found with increased homogenization pressure and total solids of the homogenized mixture was not related to the dispersed particle size. Increases in NSI also appeared unrelated to the viscosity changes brought about by homogenization at different total solids levels. Under the drying conditions used, both experimentally and under commercial conditions required by the specifications for WSDM (159), the increased solubility was retained through the drying step except in one instance where the powder had been overheated.

The slightly reduced NSI brought about by the use of emulsifier is in agreement with results reported by Fukushima and Van Buren (191) for emulsifier of HLB 14.9. Emulsifiers of HLB 7 to 10 were found to increase the NSI over the control, but the increase was slight. The authors thought that part of the insolubilization during drying might be caused by non-polar groups on the proteins; emulsifiers capable of complexing with the non-polar groups should increase redispersibility. The use of emulsifier affected the NSI so little compared to other processing variables that other factors must be involved in NSI alteration.

The mechanisms surrounding the insolubilization of soybean proteins as a result of heating and drying have been investigated (191). Insolubilization during drying occurs through intermolecular disulfide polymerization and hydrophobic interactions (192). The fact that no decrease in NSI occurred during WSDM manufacture suggests that a certain minimum drying temperature must be achieved. Homogenization
at the atomizing orifice could also serve to maintain the increased nitrogen solubility.

Wolf and Tamura (90) studied the effects of heat on the 11S protein and have concluded that heat denaturation of 11S protein at 100°C proceeds through three steps:

\[
a \quad 11S \rightarrow A \text{subunits} + (B \text{subunits})
\]

\[
\downarrow
\]

b

Soluble aggregates

\[
\downarrow
\]

c

Insoluble aggregates

The A subunits represent a 3-4S soluble fraction formed on heating while B subunits represent the part of the 11S molecule converted into aggregates. Transient appearance of a 7S fraction indicates it to be an intermediate in the reaction. 7S is postulated to be a half molecule of the 11S fraction (101) but whether the 7S fraction is the precursor for only A subunits or for both A and B subunits is not clear.

B subunits have not been identified in an unaggregated state, probably because reaction b is very rapid compared to a. The soluble aggregate (80-100 S) reaches a maximum concentration after 5 - 10 minutes of heating followed by its disappearance and precipitation on continued heating. Upon treatment with N-ethylmaleimide, which blocks sulfhydryl groups, a soluble aggregate (63S) can be identified. Treatment of the mixture with 2-mercaptoethanol (2-ME) accelerates reaction c. Since N-ethylmaleimide blocks reaction c, the sulfhydryl-disulfide interchange reaction could be responsible for the formation of high
molecular weight insoluble aggregates. The mechanism for sulfhydryl-
disulfide interchange predicts that at high concentrations of
sulfhydryl, interchange will not occur to produce aggregates (383).
However, 11S protein formed as much precipitate at high concentrations
of 2-ME as at low concentrations. The combination of heat and 2-ME
should result in a completely reduced protein, thereby exposing reactive
groups capable of interaction to form insoluble aggregates. Because of high ionic strength and the high temperatures involved, ionic
and hydrogen bonds seem unlikely; therefore hydrophobic interactions
are responsible for converting soluble aggregates to insoluble ones.

Little information is available on alteration in extractability
of the protein components in steamed soybean meal. Nash et al (193,
194) have studied the extraction of the proteins from defatted meal
and lightly steamed defatted meal with water and 2-ME. The steaming
was only carried out for 4 minutes at 100°C, equivalent to heat treat-
ment that might occur during conditioning or desolventizing of flakes
in commercial solvent extractions.

It has been well documented that the 7S and 11S proteins form
disulfide-linked polymers that are depolymerized by 2-ME (74, 76, 77,
78). Since aqueous extracts of defatted meal contain disulfide
polymers, presumably the polymers pre-exist in the meal (76). Further
polymerization of soluble polymers to insoluble forms occurs during
isolation of the proteins by precipitation (77, 78). Enhanced protein
extractability obtained by Nash et al (193) when extractions were made
with 2-ME instead of water showed that the meal contained insoluble
forms of disulfide polymers as well as soluble ones. 7S and 11S
protein fractions accounted for most of the increase in protein solubility. In contrast, the amount of 2S fraction in the extracts declined when 2-ME was used as the extractant. The 2-ME was presumed to reduce intermolecular disulfide linkages to yield the more soluble monomeric forms.

Steaming decreased the protein solubility in water (194). The insolubilization was partially reversed when 0.01 or 0.1 M 2-ME was used as extractant but the amount of protein extracted was always less than that extracted from unsteamed meal. Extraction of control meal with water yielded 54% of the meal nitrogen whereas 2-ME extracted 70 - 80%; water extracted 46% of the protein from the steamed meal compared to 65 - 68% with 2-ME.

UC analysis was used to detect disulfide polymers in the water extracts from steamed meals with and without 0.01 M 2-ME in the buffer. In the presence of 2-ME, the 15S and >15S fractions decreased significantly with an accompanying increase in 7S and 11S fractions; the shifts in area showed that soluble disulfide polymers of 7S and 15S existed in the water extracts. The amounts of soluble 7S + 11S disulfide polymers in the water extracts of steamed meals were the same as those found in control meals. Mild steaming apparently did not insolubilize the water-soluble polymers.

The authors (194) attributed increased extractability of protein from the control and steamed meals to the cleavage of intermolecular disulfide bonds in polymers of the 7S and 11S proteins. If 2-ME extracted unsteamed meals were dialyzed to remove the reducing agent, disulfide polymers of 7S and 11S reformed as shown by the increase of
15S and >15S fractions. Subtle differences were observed when the
2-ME extracts of steamed meals were dialyzed; only the 7S fraction
repolymerized when extracted with 0.01 M 2-ME; if the extraction was
made with 0.1 2-ME, neither the 7S or 11S fractions repolymerized.
7S and 11S fractions from aged meals extracted with 0.1M 2-ME also
failed to repolymerize. These results all showed that aging or mild
heating caused conformational changes in 7S and 11S proteins that
permitted 2-ME to reduce internal disulfide bonds, which, once broken,
permitted structural changes that prevented repolymerization.

No published studies have been made of protein extractability
from a fully toasted meal or flour such as is used for WSDM manufac-
ture. The results reported here clearly show by UC analysis that
homogenization increases the concentration of both 7S and 11S protein
in the water extract of the fully toasted defatted flour. The increase
in solubility of these fractions accounts for the increased NSI ob-
served. There is also an increase in the >15S fraction. The absence
of a 15S fraction might be either because this fraction was irrevers-
ibly destroyed during heat treatment of the flour or the polymer was
disrupted by homogenization or by use of 2-ME in the buffer system.
15S is considered to be a polymer of 11S (60).

In the light of the reports of Nash et al (193, 194), it seems
likely that sufficient mechanical energy is put into the system during
homogenization to break intermolecular disulfide bonds and resolubilize
the 7S and 11S fractions in the heated flour. The heat of dissociation
of the disulfide bond is 54 Kcal/mole. Walstra (384) has suggested
that energy density is the most important factor in homogenization.
From studies of the emulsification process, mechanical disruption of small fat globules can be caused only by pressure differences due to shear, turbulence and cavitation (385). Efficient globule disruption at a certain pressure depends on input energy dissipation in the shortest possible time; therefore it is a function of valve design. With a properly designed valve, eddy currents on the order of 0.1 micrometer in diameter are produced; because these currents are much smaller than the fat globules, the globules are deformed and disrupted.

A similar effect could be occurring as the soybean flour proteins pass through the homogenizer valve. Toasting of the flour caused the formation of intermolecular disulfide bonds resulting in large insoluble aggregates that could approach the micrometer size range. Passage through the valve distorted and disrupted these aggregates resulting in resolubilization. Further proof of this theory is shown by the direct dependence of NSI on the homogenization pressure in both water and whey.

Nitrogen solubility of the soybean proteins in whey is decreased, probably because of the presence of calcium in the whey salt system. However, homogenization in whey also increased NSI but the increase was not as great as that found in water.

One of the most important reactions of β-lactoglobulin, the major whey protein, is its ability to form a complex with other proteins such as κ-casein when heated. This reaction is mediated by a thiodisulfide interchange and the linkage responsible is the disulfide bond (386). In raw skim milk, β-lactoglobulin exists as a dimer; when heated to above 30°C, it dissociates into monomers. Above 55°C, it
unfolds and loses its globular structure (387); the thiol and disulfide bonds become more reactive and primary and secondary aggregation reactions can occur. During homogenization, if the soybean proteins are being resolubilized by breaking of intermolecular disulfide linkages, at the same time, under the homogenization conditions used, the thiol-disulfide linkages of β-lactoglobulin could be activated, providing the proper conditions for a protein-protein interaction between β-lactoglobulin and the 7S and 11S soybean proteins. That soybean proteins readily undergo protein-protein interactions among themselves is shown by their tendency to form gel structures when heated and concentrated (see below).

That an interaction really occurs between β-lactoglobulin and the soybean proteins under the conditions of WSDM manufacture remains to be proven. Such an interaction could occur however, leading to the formation of soluble and insoluble aggregates. As the proteins are forced through the homogenizer valve, the distance between molecules could be altered because of conformational changes brought about by the disrupting forces. If at this time, binding sites on the surfaces of the molecules or colloidal particles become newly available, interactions could readily occur, leading to aggregate formation. Aggregation by way of intermolecular disulfide linkages is characteristic of the denaturation of β-lactoglobulin regardless of the means of denaturation used because disulfide aggregates have also been formed as a result of alkali denaturation and urea denaturation (388). McKenzie et al (389) concluded from their studies that the formation of intermolecular disulfide bonds is a major factor in β-lactoglobulin complex
stability but other forms of less specific association were also occurring, perhaps involving physical entanglement of polypeptide chains. This agrees with Lyster's (390) observation that there are three forms of denatured β-lactoglobulin: one form involves disulfide interchange and the other two probably involve hydrophobic, hydrogen and ionic bonds, altering the sensitivity of β-lactoglobulin to salt and ionic strength. DellaMonica et al (391) have shown that Ca\(^{++}\) was involved in the β-lactoglobulin-casein interaction. Morr and Josephson (392) identified two whey protein-casein aggregates; one was aggregated through disulfide bonds and the second was formed by aggregation of the first by a calcium binding mechanism of some type. Data reported above for the soybean 11S protein parallel these findings in many ways.

Treatment of the soybean flour proteins in water or whey with disulfide bond reducing agents before homogenization brings about a much greater increase in NSI after homogenization than treatment of the homogenized control with reducing agents. This suggests that the conformation of the aggregated proteins or possibly the proteins themselves could be deformed by pressure change exerted at the homogenizer valve, enabling the reducing agent to penetrate into the interior to rupture critical disulfide bonds, thereby destroying the polymer and increasing solubility. Exposure of reactive portions of the molecule could then permit hydrophobic and hydrogen bonding interactions to occur, reforming the native proteins, binding other soybean proteins or whey proteins, or forming lipid-protein complexes during the homogenization process. Temperature conditions immediately following release from the homogenizer valve would favor hydrophobic bonding
which is more stable at higher temperature than either hydrogen bonds or ionic bonds (393).

Treatment of the homogenized controls in water or whey with disulfide bond reducing agents also increased NSI, showing that although some disulfide bonds, probably intermolecular ones, were present and accessible to the solvent, the homogenized proteins had stabilized into a soluble form, probably through bonds other than disulfide bonds because homogenization alone was sufficient to increase NSI.

Catsimpoolas and Meyer (394) have demonstrated that the soybean proteins will also undergo heat induced protein-lipid interactions. The inter-molecular bonding forces were considered to be non-covalent in nature. Upon homogenization, exposure of reactive surfaces in the soybean proteins other than sulfhydryls could result in lipid binding, especially since the fat globules are also being disrupted and exposing fresh surfaces as they pass through the valve. Reforming of soluble and insoluble aggregates through disulfide bonds, hydrophobic and hydrogen bonds upon cooling of the homogenized mixture could also entrap fat droplets within the aggregate structure. That disulfide bonds are involved in stabilization of the fat-protein complex in WSDM is shown by treatment of reconstituted commercial sample C with 2-ME. This sample had a very low free fat content and very little fat in the supernatant of the settled reconstituted sample. However, after treatment with 2-ME, a significant part of the fat no longer went with the settled particulates but was released into the supernatant along with solubilized protein.
The globular nature of the soybean proteins might reduce the amount of lipid bound because of structural considerations, accounting for the relative ease with which lipid is released upon input of mechanical energy during reconstitution. The random coil conformation of the caseins might present a greater surface for possible binding sites for lipid, resulting in a more concentrated and stable complex formation at lower homogenization pressures than found with the soybean proteins.

Treatment of the flour with ammonia gas before mixing also brought about an increase in NSI, probably because of the increased pH. pH effects are very important in the formation of hydrophobic bonds among molecules because of electrostatic repulsion. With higher pH, the negative charge on each molecule is increased, so mutual electrostatic repulsion is increased. Therefore hydrophobic bond formation was prevented or hydrophobic bonds were broken by homogenization, increasing the nitrogen solubility.
4. Average equivalent spherical diameter (de)

Of the three processing variables studied, only the presence of emulsifier was shown to have a significant effect ($p < .02$) on the average equivalent spherical diameter $de$ of the experimental reconstituted WSDM powders homogenized double stage. When single stage homogenization was used for processing, $de$ of the dispersed particles decreased significantly with increasing homogenization pressure, but was not affected by emulsifier or total solids of homogenization. However, the average values of $de$ were about the same regardless of whether homogenization was single or double stage, ranging from 12.30 to 13.23 micrometers for powders homogenized single stage and from 12.24 to 13.47 micrometers with one outlier of 15.10 micrometers for samples homogenized double stage.

Further proof that the processing variables studied had little effect on $de$ of the reconstituted powders homogenized double stage was shown by RSM analysis. The polynomial regression equation describing $de$ yielded a multiple correlation coefficient of only .70; the F value obtained by ANOVA showed that the correlation of the three processing variables was not significant with regard to $de$.

In contrast, not only did all three processing variables significantly ($p < .001$) affect $de$ of the concentrates homogenized double stage, there were also significant interaction of total solids with both emulsifier and homogenization pressure. None of the variables significantly affected $de$ of the concentrates homogenized single stage, however. In both cases, $de$ of the concentrates were smaller than those of the powders, ranging from 10.24 to 11.72 micrometers.
for concentrates homogenized double stage and from 10.33 to 11.65 micrometers for concentrates homogenized single stage.

In a direct comparison of de for concentrate and reconstituted powder (Table 33) the particle size of the powders increased from .25 to 1.5 micrometers over those of the concentrates as a result of spray drying. This finding is in agreement with that of Mustakas et al (143) who pointed out that spray drying reformed granular particles in their beverage that did not redisperse upon reconstitution; they implied that the particulate matter increased with increasing drying temperatures and indicated that there was no problem if drying temperatures were minimal.

Based on the findings reported here, it is theorized that the major controlling factors affecting de's of the reconstituted WSIM powders are the drying parameters, especially temperature. Some credence is lent to this theory by the de's of the commercial samples studied (Table 17). Sample A had the smallest de (13.64 micrometers) even though prepared with full fat flour; this powder was spray dried at 47% TS and an inlet air temperature of only 149°C. Drying temperatures of the other three samples are unknown; there is no maximum drying temperature listed in the specifications (159). Certainly sample B was overheated during processing, probably drying, as shown by the large de (26.31 micrometers) and by the absence of identifiable whey proteins in the electrophoretic pattern (Plate I).

Log probability plots of the average volume particle diameters of the dispersed particles of all the reconstituted experimental powders prepared without emulsifier showed a tendency to asymptote
toward an upper size limit of about 50-55 micrometers as well as
toward a lower size limit brought about by the artificial exclusion of
all particles below 8 micrometers in diameter from the count (Figure
23). The curves for the commercial samples all showed more pronounced
curvature toward a slightly higher upper size limit. Samples with
emulsifier did not show this curvature, but appeared to come close to
having a log normal distribution (Figures 14 and 23). Apparently,
the presence of emulsifier altered the distribution in such a way
that no upper size limits were evident under the experimental condi-
tions used.

Although this suggests that there may be a certain maximum
aggregate size in the reconstituted powders that would be similar for
all powders dried on the same dryer at the same temperature, it must
be pointed out that the values of de reported here are relative and
not absolute values. All measurements were made in 0.25% sodium
chloride, equivalent to .043 normal. Soybean meal proteins show a
sharp decrease in solubility with added neutral salts and, in the case
of sodium chloride, the solubility curve is minimum in the concen-
tration range of 0.05-0.10 N (97). Therefore, some aggregation of the
soybean proteins undoubtedly took place when the WSDM, reconstituted
in water to 15% TS was dispersed into the electrolyte for measurement
of de, resulting in larger particles. Since the concentration of
electrolyte was the same, the de of similar samples could have a
similar upper limit size. Because the presence of emulsifier would
alter the hydrophobicity of the solvent surrounding the protein parti-
cles, the maximum size of aggregates of the proteins in reconstituted
samples with emulsifier could also be altered, resulting in a different log probability plot.

This problem could probably have been overcome by using sweet whey dialysate as electrolyte so that the salt system concentration would be the same as that found in the reconstituted WSDM mixture. It was decided, however, because of difficulties in collecting a sufficient supply of fresh whey dialysate to conduct all necessary measurements, that relative values of de would be sufficient to determine if the three processing variables studied significantly altered the dispersed particle size.

de also appeared to be unrelated to the NSI. NSI was dependent on all three processing variables, and was not decreased by spray drying under the conditions used here (Table 33). However, NSI of WSDM can be decreased by overheating during drying as shown by the NSI (29.6%) of commercial sample B.

de did show a significant (p < .05) inverse relationship to phase separation after 24 hours of standing after reconstitution in that samples with the smaller particles (samples with emulsifier) settled more during this time period.

This finding suggests that emulsifier may be preventing the formation of lipid-protein complexes by homogenization, similar to those reported for homogenized whole milk (375). The presence of fat, adsorbed on or bound to the particulates, would alter the density, making them more buoyant, accounting for the reduced phase separation and the larger de observed in samples prepared without emulsifier.
5. Phase separation

Suspension stability after only one hour of standing after reconstitution was significantly affected by all three processing variables whereas phase separation after 24 hours of standing in the cold was significantly affected only by emulsifier and by the homogenization pressure. Settling after 24 hours of standing was shown to be linearly related both to de and to the viscosity whereas settling after only one hour of standing had no relation to the de and viscosity.

These results can be explained by assuming first of all that one hour of standing is not sufficient to wet all the WSDM components after reconstitution. In addition, the settling test required by the WSDM specifications (159) is performed in 25 ml graduated cylinders. One means of determining particle size is by gravitational sedimentation. This means that an initially uniform and relatively dense suspension of fine particles settles at a constant rate with a well-defined interface between fluid and settled particles. This form of settling is referred to as hindered settling.

Hindered settling occurs whenever there is mutual interference by the particles in their motion. Although interference can occur at low concentrations, the term hindered settling is customarily used only when the concentration is such that an observable interface exists between supernatant liquid and suspended particles. If hindered settling measurements are made in cylinders with internal diameters less than 25 mm, wall effects become important and interfere with the rate of settling of the particles (354). Therefore, since the internal diameter of the cylinders used for the WSDM one-hour settling test was...
only 20 mm, wall effects could have altered the settling rate and introduced an appreciable error in the measurements. As the 24-hour settling tests were carried out in 250 ml beakers, wall effects should not have been a factor in these measurements.

Samples prepared with added emulsifier showed greater settling over the 24-hour period even though de's of these samples were significantly smaller than controls. This can be explained on the basis of formation of some type of fat-protein complex by homogenization; emulsifier inhibits complex formation by altering the hydrophobicity of the particulates in relation to the fat droplets. Structural studies have shown that fat droplets are adsorbed to the surface of and entrapped in the crevices of proteinaceous particulates in samples with no emulsifier; settling is significantly decreased with increasing homogenization pressure, corresponding to more complex formation. The overall result would be a larger, more buoyant particle that would remain in suspension. These results suggest that suspension stability of WSDM would be enhanced by using higher homogenization pressures to increase the amount of complex formed. This is in agreement with results reported by Priepke (173) who found a significant linear relationship between the sum of pressures of all homogenizations and suspension stability of the "Illinois" soybean beverage. The minimum sum of pressures required for suspension stability was 351.6 kg/cm² if homogenization was at 32.2°C; increased homogenization temperature also enhanced suspension stability. Priepke concluded that lipid binding was partially responsible for the suspension stability of his beverage.
The enhanced suspension stability brought about by high homogenization pressure and temperature of homogenization was probably due at least in part to increased viscosity of the beverage. Best suspension stability of WSDM was found in samples with the highest viscosity on reconstitution. Therefore, it is concluded that suspension stability of WSDM is dependent on the viscosity of the reconstituted powder; this viscosity is controlled by the amount of fat-protein complex formed by homogenization of the fluid mixture. Although increased homogenization pressure and temperature would undoubtedly improve lipid-protein interaction, there is some question of how practical higher pressures would be in a commercial operation. However, revising the specifications to permit the use of the alternative processing sequence would result in a more suspension-stable product. A different type and concentration of emulsifier could also be tested for its ability to enhance complex formation under present WSDM manufacturing conditions.

6. Viscosity and gelation

The development of high viscosities during processing has been a major obstacle to the manufacture of concentrated soy milks. In addition, one of the major complaints about soybean beverages in general has been their viscous nature; the reconstituted milk analogues do not have the same mouthfeel as milk. These viscosity effects are attributed to the tendency of soybean proteins to form gel structures when heated and concentrated.

In contrast to reports for soy milk, viscosity measurements of the WSDM concentrates and reconstituted powders in this study have
shown that viscosities are generally low; the highest viscosity measured for a WSDM concentrate was only 1890 cp for the sample homogenized at 211.0-35.2 kg/cm² and 36.6% total solids without emulsifier. This suggests that the presence of whey may have altered the tendency of the soybean proteins to gel when subjected to concentration and heat.

Protein gels are formed by intermolecular interactions which produce a continuous three-dimensional network showing structural rigidity. A critical parameter in soybean protein gelation is the protein concentration; below 8% total solids, aggregation and some viscosity increase are observed, but no gelation (79). Apparently some overlap of functional groups on the proteins or their dissociated subunits is necessary for gel network formation.

Catsimpoolas and Meyer (395) have proposed that gelation of the soybean globulins is accomplished by heating and subsequent cooling of the protein dispersions according to the following scheme:

\[
\begin{array}{c}
\text{sol} \xrightarrow{\text{heat}} \text{Progel} \xrightarrow{\text{heat}} \text{gel} \\
\quad \downarrow \text{cool} \quad \downarrow \\
\text{excess heat} \quad \text{heat} \\
\quad \downarrow \\
\text{metasol}
\end{array}
\]

The dispersion (sol) is converted to a progel (characterized by high viscosity) by heat and then sets to a gel of higher viscosity on cooling. The activation of the sol to the progel is irreversible in that the quaternary structure of the soybean globulin has been disrupted by
heat. Irreversible thermal disruption of the 11S globulin and dissociation into subunits has been documented (90). Dissociation of the soybean protein molecules results in exposure of groups capable of hydrophobic bonding; the importance of hydrophobic bonds in stabilization of soybean globulin structure has also been shown (17, 75). Therefore hydrophobic interactions of the non-polar groups to form an associated structural network could be involved in conversion of the sol to the progel because formation of these bonds is favored by increasing temperature (393). Upon cooling of the progel and subsequent formation of the gel, electrostatic cross links and hydrogen bonding surrounded by water could be responsible for the further increase in viscosity because this transition is reversible and these two types of bonds are favored by lowering temperature.

A reversible heat induced dissociation of the $\alpha_{sl}$-k-casein complex has been demonstrated which occurred between 45 and 54°C (396). The dissociation was attributed to a transition from predominantly hydrophilic interactions (hydrogen bonds) between the two proteins at low temperature to hydrophobic interactions involving only k-casein at elevated temperature.

Upon overheating of the progel (or gel) a metasol is formed which does not gel on cooling (79). Catsimpoolis and Meyer (395) found that this conversion was accompanied by protein degradation since ammonia release was observed. The conversion of asparagine and glutamine residues to carboxylate groups by release of ammonia could convert the protein to a non-gelling one. This type of modification introduces electrostatic repelling forces and would inhibit hydrogen
bonds formation between amide groups.

Disulfide bond reducing agents reduce viscosity and inhibit gelation of soybean proteins in low concentration (79); at higher concentrations (10% 2-mercaptoethanol) however, gelation was enhanced. Although Circle et al interpreted the results as being indicative of disulfide bond participation in the gelation process, Catsimpoolas and Meyer suggested a different interpretation. Cleavage of intermolecular disulfide bonds at low reagent concentration depressed gelation, but at high concentrations, cleavage of intramolecular disulfide bonds occurred. This would facilitate disruption of the quaternary structure and exposure of reactive groups due to dissociation and unfolding of the subunits, resulting in increased viscosity and gel strength through other types of bonds.

Treatment of the defatted toasted soybean flour proteins with disulfide bond reducing agents reduced the viscosity on homogenization in whey; initial viscosities in whey were about the same as the control. In water, however, initial viscosities of the treated unhomogenized samples were both slightly greater than the control; after homogenization, viscosity of the sample treated with sodium bisulfite was greater than that of the control but viscosity of the 2-ME treated sample was less. Viscosity increases in all cases were slight, probably because concentration of the soybean globulins was low.

Alteration in pH by fermentation of the whey had little effect on viscosity of the soybean flour proteins after homogenization until the pH of the whey fell below 5, whereupon viscosities of the homogenized samples were decreased. Catsimpoolas and Meyer (395) showed
that pH had a marked effect on gel formation. The progel and gel formed by cooling the system after heating were dependent on hydrogen and electrostatic cross links; at acidic pH, the carboxyl groups of the soybean proteins are less ionized, so interaction is weakened, reducing the viscosity.

Guy et al (154) observed that the viscosity of the soy flour dispersion was considerably reduced when whey was added to the mixture; they attributed this to the presence of an interfacially active material present in the whey. However, Catsimpoolas and Meyer (395) showed that at temperatures above 70°C, the viscosities of the soybean globulin progel and gel decreased with increasing ionic strength; below this temperature, higher viscosity was favored by higher salt concentration. Although these results suggest the participation of ionic bonds in the gelling process, the soybean globulins may be more stable to heat in the presence of high salt concentrations; the dissociation of the proteins into subunits is inhibited. Therefore, as 70°C is in the vicinity of HTST pasteurizing temperatures generally used for WSDM manufacture, the whey salt system could be responsible for the inhibition of viscosity buildup during the pasteurizing and condensing operations. Entirely different results might be obtained if batch pasteurization of the fluid WSDM mixture at 26% total solids were carried out at 63°C for 30 minutes, especially since whey proteins also undergo gelation at temperatures as low as 60°C (296).

Although there are some differences of opinion, most workers are agreed that homogenization increases the viscosity of whole milk (168). Variations in homogenization pressure and temperature of
homogenization probably account for the discrepancies observed. The percentage of fat in the milk affects viscosity after homogenization, but this effect was not great unless the fat content was increased to 5 to 6% (397, 398); no increase in viscosity occurred when skim milk was homogenized (399).

Milk homogenized single stage under high pressure (140.6-281.3 kg/cm²) is subject to fat globule clustering, a tendency which increases with increasing fat content (399). The appearance of these clusters is responsible for the increase in viscosity observed (400). A second low pressure stage, usually 35.2 kg/cm², breaks up the clusters so a lower viscosity is found even though the homogenization level is virtually unchanged (239). This behavior has been attributed to denaturation of the creaming factor (immunoglobulins in the plasma protein) by homogenization. Part of the non-clustering characteristics of homogenized milk could be attributed to the nature of the altered fat globule surface membrane which contains adsorbed plasma proteins (400).

In contrast to results for the effects of single and double stage homogenization on the viscosity of whole milk concentrates (239), WSDM concentrates homogenized single stage did not have higher viscosities than concentrates homogenized double stage at about the same total solids levels. This suggests that clustering is not occurring because, in the whey-soybean system, an entirely different type of membrane from the milk fat globule membrane would be formed on the surfaces of the dispersed soybean oil globules as a result of homogenization.
Catsimpoolas and Meyer (394) demonstrated that certain protein-lipid interactions brought about by heating can increase viscosity and enhance gelation of soybean globulins. Viscosities of both unheated and heated dispersions increased with increased concentration of triglyceride. The shorter the chain length, the higher the viscosity. Mono- and di-glycerides also resulted in higher viscosities in the gelled state than triglycerides. Increased hydrogen bonding could account for the results. Saturated fats also produced higher viscosities than unsaturated ones. Phosphatides and cholesterol also enhanced gelation; in this case, hydrogen bonds are formed during cooling between the polar groups of the phosphatides and side chain groups of the proteins. Effects of cholesterol were attributed to hydrogen bonds or van der Waals forces.

Fox et al (375) showed that the fat globule membrane is not necessary for the formation of the fat-protein complex as a result of homogenization of whole milk. Further evidence that a fat-protein complex is being formed by homogenization of the whey-soy protein is suggested by the fact that the reconstituted WSDM control powders showed significant increases in viscosity with increased homogenization pressure and total solids of the mixture homogenized, whereas viscosities of the reconstituted powder samples formulated with emulsifier were all about the same except for the sample homogenized at 211.0-35.2 kg/cm² and 40% total solids. When Whitnah et al (401) homogenized milk containing 4% fat at pressures ranging from 1.05 to 246.1 kg/cm² and temperatures of 4 to 49°C, viscosity increased in all cases; the increase was independent of temperature but increased
at higher homogenization pressures. Whitnah et al suggested that changes in the viscosity of milk as a result of homogenization are independent of changes in the fat particle size. Brunner (400) attributes these viscosity increases to newly formed protein-lipid complexes; unfortunately, Fox et al (375) did not attempt to correlate viscosity of their homogenized milks with the amount of complex formed. However, formation of a fat-protein complex in the whey-soy system would account for the increase in viscosity observed in the control samples whereas emulsifier, under the conditions of the experiment, inhibits complex formation.
A. Tests commonly used to characterize initial rehydration properties of milk powders, could also be used with slight modifications to evaluate WSDM.

B. Initial rehydration characteristics of WSDM were similar to those of milk powders, but after reconstitution, properties similar to those of soy beverages became apparent, making it necessary to characterize WSDM further. Three additional tests, the NSI, measurement of average equivalent spherical diameter de of the dispersed particles, and phase separation after reconstitution were successfully used for this purpose.

C. Emulsifier had an adverse effect on the rehydration properties. Its presence destabilized the fat emulsion during the drying process, resulting in increased free fat content and reduced dispersibility of the powder and increased phase separation and reduced NSI of the reconstituted powder.

D. RSM analysis of the effects of the three processing variables on five of the rehydration properties showed that only the NSI could be accurately predicted from the regression equations. This meant that under the conditions of the experiment, the processing variables selected were responsible for most of the variation in NSI.

E. de was not the sole factor involved in phase separation after reconstitution. Phase separation was related to both de and the
viscosity of the reconstituted powder; viscosity was significantly
affected by all the processing variables.

F. Formation of a fat-protein complex by homogenization was respon-
sible for increased de and viscosity and improved suspension stability;
it’s formation was inhibited by emulsifier. Bonds were probably hydro-
phobic in nature and could be broken by input of mechanical energy
into the system during reconstitution resulting in release of fat
globules from the particulates.

G. Homogenization increased NSI and the increase was maintained
through the drying stage. The increase in NSI was due to resolubili-
ization of the 7S and 11S fractions of the soybean globulins. The
input of energy at the homogenizer valve was sufficient to break the
intermolecular hydrophobic and disulfide bonds formed during heat
treatment of the flour, resolubilizing the proteins.
LIST OF REFERENCES


(364) Barr, Goodnight, Sall and Helwig. SAS Institute, Inc., Box 10066, Raleigh, N. C. 27605.

(365) Foulk, C. W., H. V. Moyer and W. M. MacNevin, Laboratory Procedures: Quantitative Chemical Analysis. The Ohio State University, Columbus, Ohio, p. 73, 1949.


APPENDIX A

Calculations of Particle Size Distributions

Particle size distributions of the experimental WSDM powders were measured by light microscopy with an image splitting eyepiece. Sorting of the particles into size classes and the subsequent calculations of Martin's diameters were carried out by computer; a sample printout is shown in Table U6. The sample data listed are those for the experimental WSDM powder processed with double stage homogenization at 1800-500 psi (126.6-35.2 kg/cm²) and 40% total solids without added emulsifier.

To determine if a sufficient number of particles were measured to yield meaningful results, $d_1$ was calculated after every 50 particles and results plotted against the number of particles measured as shown in Figure 70. The point where $d_1$ changes very little with increasing numbers of particles measured indicates the minimum number of particles it is necessary to measure (196). Even though in this case, Figure 70 shows that measurement of 350 particles was probably sufficient, the data shown in Table U6 represent measurement of 602 particles, because, the greater the number of particles in each size class, the more accurate the results; each size class should contain at least 10 particles (349). As shown in Table U6, the few particles to be found in the size categories above 40 micrometers indicate that more than 600 particles should have been measured for best results.
Table 46. Calculations for Martin's Diameters

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<th>Number of particles in this size category</th>
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<th>( nd^3 )</th>
<th>( nd^4 )</th>
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Sum = 602 \( \sum \) 8162 \( \sum \) 165682 \( \sum \) .45308E07 \( \sum \) .151444E09

\( \bar{d}_1 = 13.558 \quad \bar{d}_2 = 20.299 \quad \bar{d}_3 = 27.346 \quad \bar{d}_4 = 33.425 \)

\( \bar{d} = 16.589 \quad \bar{D} = 19.597 \)
Variation of $d_1$ with number of powder particles measured.

Figure 70
Operator fatigue usually limits the total number of particles measured by microscopy.

The size frequency percentages and the cumulative frequency percentages are tabulated in Table 147. The cumulative size frequency percentages may be used to construct a histogram of the particle size data such as the one shown in Figure 71. The histogram is a bar graph where the bottom of each bar is the width of the corresponding size class. It is evident from Figure 71 that the bulk of the particles fell between 4 and 20 micrometers in diameter.

The cumulative percentage distributions of the products \( nd, nd^2 \), and \( nd^3 \) may be used to construct the diagram shown in Figure 72. These curves show the relationship between size, surface and volume and are plotted so as to show the cumulative percentages less than the stated size.

A log probability graph for the cumulative upward sum of \( nd^3 \) is shown in Figure 73. Although summation may be in either direction, the upward sum is usually used so that data may be plotted as the percentage greater than the stated size.

The log probability graph is a convenient devise for representing the cumulative curve as a straight line and is a means of determining if the distribution follows the log normal law. Log normal distribution functions may be completely described by the two parameters \( \bar{d} \), the geometric mean diameter and \( \sigma \), the geometric standard deviation. These values may be obtained directly from the log probability graph, provided the particle size distribution obeys the log normal distribution function and yields a straight line. The value of \( \bar{d} \) is equal
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<th>Cum. %</th>
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<td>4.09</td>
<td>99.8</td>
<td>4.09</td>
<td>6.97</td>
</tr>
</tbody>
</table>

Table 47. Cumulative Size Frequencies
Size frequency distribution histogram.
(2 micrometer intervals)

Figure 71
Relationship between particle size (nd), surface (nd²) and volume (nd³) in terms of cumulative frequency.

Figure 72
Log probability plot of particle size distribution of an experimental WSDM powder.

Figure 73
to the 50% value of the distribution and $\sigma$ is equal to the value of
the size at 50% probability divided by that at 15.37% or the value at
84.13% probability divided by that at 50% (354). Because the line
shown in Figure 73 is curved, the distribution is not log normal. The
curve is asymptotic toward an upper size limit which means that the
upper limit has a finite size whereas for a log normal distribution
the upper size limit should approach infinity (196).
APPENDIX B

Calculations of Coulter Counter Data

A sample computer printout for Coulter Counter data for the experimental WSDM sample homogenized double stage at 1800-500 psi (126.6-35.2 kg/cm²) and 40% total solids without emulsifier is shown in Table 48. The use of an automatic counting devise resulted in the counting of a very large number of particles, 92,502 in this case. Because it is particle volume that is measured directly by the Coulter Counter, the upward sum of the cumulative weight percentages of nd³ was computed in order to plot the size distribution on log probability paper.

The log probability plot obtained is shown in Figure 74. This plot appears very close to a straight line; however, the geometric mean diameter d₃₃, read from the graph, is only 14.8 micrometers compared to the calculated value of 16.7 micrometers (Table 48).

The shape of the curve clearly matches the case whereby a modification in the log normal distribution occurs because of the exclusion of particles below a certain size, in this case 3 micrometers (196). This exclusion occurred due to limitations in the Coulter Counter equipment used for the measurements.
Table 48. Computer Printout of Calculations of Coulter Counter Data

<table>
<thead>
<tr>
<th>Counts</th>
<th>n</th>
<th>diameter micrometers</th>
<th>n</th>
<th>Average diameter micrometers</th>
<th>nd</th>
<th>nd²</th>
<th>nd³</th>
<th>nd⁴</th>
<th>Cumulative wt. Per Cent</th>
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<td>92502</td>
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<td>.17404E09</td>
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Table 48 (Continued)

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<th>Average diameter micrometers</th>
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<th>nd²</th>
<th>nd³</th>
<th>nd⁴</th>
<th>Cumulative wt. Per. Cent</th>
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<td>.53736E07</td>
<td>.22838E08</td>
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<td>45</td>
<td>9</td>
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<td>.22500E05</td>
<td>.11250E07</td>
<td>.56250E08</td>
<td>.55</td>
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<tr>
<td>6</td>
<td>55</td>
<td></td>
<td></td>
<td>sums  =</td>
<td></td>
<td></td>
<td></td>
<td>.10906E07</td>
</tr>
</tbody>
</table>

\[
\bar{d}_1 = 11.79 \quad \bar{d}_2 = 12.91 \quad \bar{d}_3 = 14.50 \quad \bar{d}_4 = 16.66 \quad \overline{A} = 12.34 \quad \text{de} (\overline{d}) = 13.02
\]
Log probability plot of particle size distribution of dispersed particles of an experimental WSDM powder.

Figure 74
APPENDIX C

Samples Processed with Single Stage Homogenization

1. Analytical Results

Properties of the WSDM powders processed with single stage homogenization at 12.6 and 29\% total solids (TS) are tabulated in Table 49. Examination of the data show a considerable variation in the TS of the concentrates that were spray dried. Powders prepared without emulsifier and dried above 40\% TS had moisture contents higher than that permitted by specifications (3.25\%) (159); the presence of emulsifier in the fluid mixture appeared to decrease the moisture content of the finished powder, even when the TS of the concentrate to be dried was as high as 46\%.

Minimum protein and fat contents for WSDM permitted by specifications are 19.0\% for both; all samples met the required specifications.

Results for the other properties generally paralleled those reported for the samples prepared with double stage homogenization (Table 18). As before, emulsifier seemed to have the greatest effect on the properties.

NSI values ranged between 34.6 and 39.6\%, considerably lower than values observed for three of the commercially prepared samples and the experimental samples processed with double stage homogenization (Tables 14 and 18).
Table 49. Properties of Experimental WSDM Samples Homogenized Single Stage at 12.6 and 29% TS

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>Total Solids of Homo. %</th>
<th>Total Solids of Spray Drying %</th>
<th>Protein (Total N x 6.25) %</th>
<th>NSI</th>
<th>Total Fat %</th>
<th>% of Total that is Free Fat</th>
<th>% of Total Fat in Soluble Nitrogen Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>126.6</td>
<td>12.6</td>
<td>41.3</td>
<td>3.58</td>
<td>20.08</td>
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<td>19.2</td>
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</tr>
<tr>
<td>175.8</td>
<td>12.6</td>
<td>44.8</td>
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<td>20.14</td>
<td>37.3</td>
<td>20.3</td>
<td>18.0</td>
</tr>
<tr>
<td>211.0</td>
<td>12.6</td>
<td>43.9</td>
<td>4.22</td>
<td>19.30</td>
<td>39.4</td>
<td>21.7</td>
<td>21.2</td>
</tr>
<tr>
<td>126.6E*</td>
<td>12.5</td>
<td>45.5</td>
<td>2.89</td>
<td>20.44</td>
<td>36.7</td>
<td>19.8</td>
<td>28.4</td>
</tr>
<tr>
<td>175.8E</td>
<td>12.7</td>
<td>45.1</td>
<td>2.02</td>
<td>19.81</td>
<td>38.1</td>
<td>21.4</td>
<td>34.9</td>
</tr>
<tr>
<td>211.0E</td>
<td>12.5</td>
<td>46.4</td>
<td>3.26</td>
<td>19.98</td>
<td>37.4</td>
<td>22.2</td>
<td>41.9</td>
</tr>
</tbody>
</table>

-----------------------12.6% TS Homogenized-----------------------

| 126.6                          | 28.4                    | 35.1                          | 3.07                        | 20.03 | 37.9        | 20.3                        | 22.5                         | 92.7                             |
| 175.8                          | 29.0                    | 42.7                          | 3.44                        | 19.78 | 39.4        | 20.2                        | 16.8                         | 95.1                             |
| 211.0                          | 29.0                    | 39.7                          | 3.04                        | 19.35 | 39.4        | 22.9                        | 21.4                         | 91.6                             |
| 126.6E*                        | 29.1                    | 38.3                          | 2.02                        | 21.20 | 34.6        | 20.9                        | 53.2                         | 96.5                             |
| 175.8E                         | 28.9                    | 38.9                          | 2.58                        | 21.48 | 33.3        | 21.0                        | 45.8                         | 92.8                             |
| 211.0E                         | 29.0                    | 39.8                          | 2.23                        | 20.63 | 36.4        | 22.2                        | 49.2                         | 92.2                             |

*E = 0.5% emulsifier added
<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>ADMT Solubility Index (ml)</th>
<th>Bulk Density g/cc</th>
<th>Sinkability %</th>
<th>Dispersibility %</th>
<th>Phase Separation 1 hour ml</th>
<th>24 hours %</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>126.6</td>
<td>4.5</td>
<td>.42</td>
<td>76.8</td>
<td>97.2</td>
<td>19.5</td>
<td>70.8</td>
<td>6.62</td>
</tr>
<tr>
<td>175.8</td>
<td>4.7</td>
<td>.45</td>
<td>94.6</td>
<td>84.6</td>
<td>19.0</td>
<td>65.3</td>
<td>6.59</td>
</tr>
<tr>
<td>211.0</td>
<td>4.2</td>
<td>.45</td>
<td>100</td>
<td>81.1</td>
<td>19.5</td>
<td>68.0</td>
<td>6.62</td>
</tr>
<tr>
<td>126.6E*</td>
<td>5.0</td>
<td>.44</td>
<td>100</td>
<td>81.2</td>
<td>18.2</td>
<td>68.1</td>
<td>6.60</td>
</tr>
<tr>
<td>175.8E</td>
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<td>.39</td>
<td>100</td>
<td>83.5</td>
<td>18.5</td>
<td>69.4</td>
<td>6.64</td>
</tr>
<tr>
<td>211.0E</td>
<td>5.0</td>
<td>.42</td>
<td>100</td>
<td>82.0</td>
<td>18.0</td>
<td>67.1</td>
<td>6.60</td>
</tr>
</tbody>
</table>

*E = 0.5% emulsifier added
The presence of emulsifier resulted in undesirably high levels of free fat; the 6 controls also contained higher levels of free fat than are normally found in spray dried whole milk powders (3-14%) (176). In contrast to powders processed double stage, increasing the homogenization pressure seemed to have little effect on the free fat content of the finished powder; however, free fat content is greatly influenced by drying parameters such as TS of the concentrate fed to the dryer (201). Therefore, any effects of homogenization on free fat content may be obscured by the effects of variation in the dryer feed TS, even though no consistent effects of feed TS on free fat content could be identified in these samples.

Results for the percentage of fat in the soluble nitrogen fraction were variable; the bulk of the lipid appeared to be in the supernatant, suggesting that single stage homogenization under the pilot plant conditions used resulted in little lipid binding.

Measurement of the American Dry Milk Institute solubility index showed that samples containing 0.5% emulsifier were less soluble than their controls; similar results were found with the powders processed double stage. Increased homogenization pressure resulted in improved solubility.

Bulk densities of the powders showed considerable variation. However, this also could be accounted for by the variability in the TS spray dried; highest bulk densities were found in those samples spray dried at the highest TS levels (samples homogenized at 12.6% TS). All samples conformed to density requirements in the specifications, however (.32 to .53 g/cc).
As with the powders processed double stage, the presence of emulsifier sharply increased sinkability and reduced dispersibility. The greatest decrease in dispersibility was observed in the samples homogenized at 29% TS. The high moisture contents of the two samples prepared without emulsifier and homogenized at 12.6% TS at pressures of 175.8 and 211.0 kg/cm² may be responsible for their reduced dispersibilities.

The data for phase separation after reconstitution show that the suspension stabilities of these samples were very poor. Specifications require that the supernatant liquid after standing for one hour at room temperature not exceed 10 ml; the results show that none of the samples met specifications for suspension stability (159). The presence of emulsifier appeared to improve suspension stability slightly, especially in those samples homogenized at 29% TS. After 24 hours of standing, the presence of emulsifier seemed to have little effect on the suspension stability of the samples homogenized at 12.6% TS. Because the pH of all samples was about the same, pH was eliminated as a factor in the solubility and phase separation measurements as with the powders processed with double stage homogenization.

Brookfield viscosity measurements on the reconstituted powders (15% TS) were used to generate the flow curves shown in Figures 75, 76, 77 and 78. Comparison of the flow curves for both sample sets showed that homogenization pressure had little effect on the viscosity; viscosity seemed to be slightly higher in those samples homogenized at 29% TS. Comparison of the flow curves for samples containing emulsifier to those of the controls showed that emulsifier had no effect on
$A = 126.6 \text{ kg/cm}^2 \quad B = 175.8 \text{ kg/cm}^2 \quad C = 211.0 \text{ kg/cm}^2$

Viscosity flow curves for reconstituted powders homogenized single stage at 12.7% total solids.

Figure 75
A = 126.6 kg/cm²  B = 175.8 kg/cm²  C = 211.0 kg/cm²

Viscosity flow curves for reconstituted powders homogenized single stage at 12.7% total solids with 0.5% emulsifier.

Figure 76
A = 126.6 kg/cm²  B = 175.8 kg/cm²  C = 211.0 kg/cm²

Shear Rate

Viscosity flow curves for reconstituted powders homogenized single stage at 29.0% total solids.

Figure 77
\[ A = 126.6 \text{ kg/cm}^2 \quad B = 175.8 \text{ kg/cm}^2 \quad C = 211.0 \text{ kg/cm}^2 \]

Viscosity flow curves for reconstituted powders homogenized single stage at 29.0% total solids with 0.5% emulsifier.

Figure 78
the viscosity of samples homogenized at 29% TS, and decreased viscosity only slightly in those samples homogenized at 12.7% TS. Shapes of the flow curves were similar to those generated for the commercially prepared samples and for the samples processed with double stage homogenization (Figures 9, 15-20) but viscosities were lower.

A summary of the average particle diameters for the 12 powder samples is listed in Table 50. Data were calculated as shown in the example given in Appendix A. Examination of the data showed considerable variation especially in the geometric mean $\bar{d}_4$ probably due to variation in the TS of the concentrates that were spray dried. However, results for the average volume diameter $\bar{D}$ show more consistency; the greater average diameters are found in those samples homogenized at 12.7% TS that were dried at the higher concentrate TS levels. Of those samples homogenized at 29% TS, the greatest value of $\bar{D}$ (25.57 micrometers) was found in the sample 175.8 kg/cm$^2$ dried at 42.7% TS.

One value of $\bar{D}$ in the set of samples homogenized at 12.7% TS was much higher (36.62 micrometers) than all the rest. Examination of the raw counting data for this sample (homogenized at 175.8 kg/cm$^2$ with no emulsifier) showed the presence of several particles 100 micrometers in diameter and larger; as this sample also had the highest moisture content (4.52%), the very large particles probably represented aggregates or agglomerates of several smaller particles. The largest particle diameter measured both in the remaining samples of this set and in the samples homogenized at 29% TS was 90 micrometers (homogenized at 126.6 kg/cm$^2$, 29% TS, with emulsifier).
Table 50. Average Particle Diameters of Powders
Homogenized Single Stage

<table>
<thead>
<tr>
<th>Homogenization Pressure (kg/cm²)</th>
<th>$d_1$</th>
<th>$d_2$</th>
<th>$d_3$</th>
<th>$d_4$</th>
<th>$\Delta$</th>
<th>$\bar{D}$</th>
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<td>126.6</td>
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<td>40.45</td>
<td>19.74</td>
<td>23.35</td>
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<td>175.8</td>
<td>25.55</td>
<td>35.65</td>
<td>53.92</td>
<td>83.50</td>
<td>30.18</td>
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<td>211.0</td>
<td>19.86</td>
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<td>37.41</td>
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<td>20.61</td>
<td>23.25</td>
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<tr>
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<td>18.69</td>
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</table>

29.0% TS homogenized

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<th>$d_2$</th>
<th>$d_3$</th>
<th>$d_4$</th>
<th>$\Delta$</th>
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<td>33.05</td>
<td>18.83</td>
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<td>15.43</td>
<td>25.65</td>
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<td>62.24</td>
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<td>25.57</td>
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<td>28.55</td>
<td>34.90</td>
<td>19.59</td>
<td>22.21</td>
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</table>

*E = 0.5% emulsifier added
Log probability plots of the particle size distributions for the 12 powders all took the general form shown in Figure 79, representing data for the powder samples homogenized at 126.6 kg/cm² without emulsifier, with the exception of the sample homogenized at 175.8 kg/cm² at 12.7% TS without emulsifier; data for this sample are shown in Figure 80. Because the plots are non-linear, the data apparently follow a modified log normal distribution. The curve in Figure 79 is asymptotic toward an upper size limit. This curve and those for 10 of the 11 other samples has the same general shape as that of the commercially prepared WSDM sample A (Figure 10).

The general shape of the curve in Figure 80 indicates a definite bimodality in the distribution which could be caused by powder aggregation in the dryer after atomization or by agglomeration after drying caused by the high moisture content. This curve has the same shape as those for the commercially prepared samples B, C and D (Figures 11, 12 and 13).

The average equivalent spherical diameters (de) for the dispersed particles of the 12 reconstituted powder samples are tabulated in Table 51. Data are given in micrometers for the de above 8 micrometers. The data were calculated as shown by the example given in Appendix B. Examination of the data for both sample sets suggests that the presence of emulsifier may result in a slightly smaller particle when the samples are homogenized at the same pressure; similar results were found with the powders processed with double stage homogenization. Increasing homogenization pressure also appears to reduce the particle size; this is shown in the decrease in weight
Log probability plot of particle size distribution of experimental WSDM powder homogenized at 126.6 kg/cm² and 12.7% total solids.

Figure 79
Log probability plot of particle size distribution of experimental WSDM powder homogenized at 175.8 kg/cm² and 12.7% total solids.

Figure 80
Table 51. Average Equivalent Spherical Diameter de of Reconstituted WSDM Powders Homogenized Single Stage

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>de &gt;8 micrometers</th>
<th>Cumulative weight per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>126.6</td>
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<td>86.7</td>
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<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>de &gt;8 micrometers</th>
<th>Cumulative weight per cent</th>
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<tr>
<td>126.6</td>
<td>13.06</td>
<td>86.0</td>
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<tr>
<td>175.8</td>
<td>12.62</td>
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<td>12.54</td>
<td>84.0</td>
</tr>
<tr>
<td>126.6E*</td>
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<td>84.3</td>
</tr>
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<td>12.77</td>
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</tr>
<tr>
<td>211.0E</td>
<td>12.47</td>
<td>83.0</td>
</tr>
</tbody>
</table>

*E = 0.5% emulsifier added
per cent with increasing homogenization pressure and is especially clear in those samples homogenized without emulsifier. The TS of homogenization appeared to have little if any effect on the particle size, however.

Log probability plots were prepared for the particle size distributions for the 12 reconstituted samples; curves for all samples had the forms shown in Figure 81 for the samples homogenized at 12.7% total solids and 126.6 kg/cm², both with and without emulsifier. Many of the curves for these samples, in contrast to the data for the commercially prepared samples (Figure 14) were close to being linear in form, especially those samples prepared with emulsifier, indicating that the distribution is very close to log-normal.

2. Statistical Evaluation by ANOVA

The effects of the three processing variables on some of the properties of the 12 WSDM samples were evaluated for significance by analysis of variance (ANOVA). Properties analyzed were the moisture content of the powders, the sinkability, dispersibility, NSI, free fat, per cent of fat in the soluble nitrogen fraction, settling after both one hour and 2¼ hours of standing after reconstitution, the Brookfield viscosity at 30 rpm, the average volume particle diameter D of the powders and the average equivalent spherical diameter de of the reconstituted samples. The results are tabulated in Table 52.

Examination of the results showed that the presence of the emulsifier had a significant effect on six of the 11 properties examined. Dispersibility, NSI, moisture content and settling after one hour of standing after reconstitution were all significantly decreased by the
Log probability plots of dispersed particles of experimental WSDM powder homogenized at 126.6 kg/cm² and 12.7% total solids.

Figure 81
Table 52. ANOVA for WSDM Powder Samples Homogenized Single Stage

A = TS  B = Emulsifier  C = HP  AB = TS:Emulsifier  AC = TS:HP

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
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<td>3.933</td>
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<td></td>
<td>C</td>
<td>.204</td>
<td>.102</td>
<td>.478</td>
<td>.6390</td>
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<td></td>
<td>Pooled</td>
<td>1.497</td>
<td>.214</td>
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<table>
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<th>Level</th>
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<td>1652.053</td>
<td>14.214</td>
<td>.0070**</td>
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<td>.489</td>
<td>.6325</td>
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<td>402.521</td>
<td>12.431</td>
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<td>22.102</td>
<td>.683</td>
<td>.536</td>
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<td>14.279</td>
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<td>.613</td>
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<td>43.390</td>
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<td>.505</td>
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<td>0.000</td>
<td>0.000</td>
<td>.000</td>
<td>1.000</td>
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429
Table 52 (Continued)

7. Settling one hour

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<th>Level</th>
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<td>Total</td>
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<td>A</td>
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<td>1.505</td>
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<td>9.630</td>
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<td>.328</td>
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<td>.439</td>
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Error

8. Settling 24 hours

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<td>17.280</td>
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<td>5.432</td>
<td>2.716</td>
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<td>.620</td>
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<td>Pooled</td>
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<td>37.145</td>
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Error

9. Viscosity

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<td>A</td>
<td>1</td>
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<td>.238</td>
<td>6.531</td>
<td>.0378*</td>
</tr>
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<td>.022</td>
<td>.022</td>
<td>.595</td>
<td>.466</td>
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<td>C</td>
<td>2</td>
<td>.0036</td>
<td>.0013</td>
<td>.050</td>
<td>1.000</td>
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<tr>
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Error

10. Average Particle Diameter

<table>
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<td>Total</td>
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<tr>
<td>B</td>
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<td>29.925</td>
<td>2.194</td>
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Error

11. Average Equivalent Diameter

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</tr>
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<td>.012</td>
<td>.217</td>
<td>.656</td>
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<td>.104</td>
<td>1.882</td>
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</tr>
<tr>
<td>C</td>
<td>2</td>
<td>.827</td>
<td>.414</td>
<td>7.446</td>
<td>.0185*</td>
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<tr>
<td>Pooled</td>
<td>7</td>
<td>.389</td>
<td>.056</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Error
emulsifier whereas sinkability and free fat content were increased.

Total solids of the homogenized mixture had no significant effect on any property except moisture content and viscosity. Moisture content was significantly lower in those samples homogenized at 29% TS; however, as the TS of the concentrates fed to the spray dryer were lower, this is undoubtedly the reason for the lower moisture contents in the powders. Brookfield viscosities at 30 rpm were significantly higher in the powders homogenized at 29% TS; data used for ANOVA are tabulated in Table 53. This can also be seen from a comparison of the flow curves for the two sample sets shown in Figures 75-79.

Increased homogenization pressure had a significant effect only on de; de was reduced as homogenization pressure increased. There was no significant reduction in phase separation either after one hour or 24 hours of standing brought about by increased homogenization pressure, however.

None of the processing variables investigated had any effect on the average volume particle diameter $\bar{D}$; this would probably be expected because of the wide variation in the results caused by the range of TS in the concentrates feeds to the dryer.

No significant AB (TS·emulsifier), AC (TS·HP) or BC (emulsifier·HP) interaction effects were observed for any of the properties examined.

3. Additional Experiments

The power inputs to the homogenizer required to homogenize the WSDM concentrates single stage at two total solids levels (14.0 and 28.6%) are tabulated in Table 54 along with the ANOVA of the data. As with the samples homogenized double stage, the power input increased
Table 53. Brookfield Viscosities at 30 rpm of Reconstituted WSDM Powders Homogenized Single Stage

<table>
<thead>
<tr>
<th>Homogenization</th>
<th>Pressure (kg/cm²)</th>
<th>Brookfield Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.6% TS homogenized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>126.6</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td>175.8</td>
<td>2.90</td>
<td></td>
</tr>
<tr>
<td>211.0</td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td>126.6E*</td>
<td>2.59</td>
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</tr>
<tr>
<td>175.8E</td>
<td>2.47</td>
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<tr>
<td>211.0E</td>
<td>2.88</td>
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<tr>
<td>29% TS homogenized</td>
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<td>126.6</td>
<td>3.14</td>
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<td>175.8</td>
<td>3.06</td>
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<tr>
<td>211.0</td>
<td>2.84</td>
<td></td>
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<tr>
<td>126.6E*</td>
<td>2.83</td>
<td></td>
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<tr>
<td>175.8E</td>
<td>3.19</td>
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<tr>
<td>211.0E</td>
<td>3.08</td>
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</table>

*E = 0.5% emulsifier added
Table 54. Significance by ANOVA of Power Input Required to Homogenize WSDM Concentrates Single Stage at Two Total Solids Levels

<table>
<thead>
<tr>
<th>Homogenization Pressure kg/cm²</th>
<th>TS of Power homo. %</th>
<th>Power input amps.</th>
<th>TS of Power homo. %</th>
<th>Power input amps.</th>
</tr>
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<tbody>
<tr>
<td>126.6</td>
<td>13.7</td>
<td>11.4</td>
<td>28.2</td>
<td>12.0</td>
</tr>
<tr>
<td>175.8</td>
<td>14.0</td>
<td>12.2</td>
<td>28.5</td>
<td>13.8</td>
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<td>211.0</td>
<td>14.4</td>
<td>14.0</td>
<td>28.4</td>
<td>13.6</td>
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<tr>
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<td>14.0</td>
<td>11.5</td>
<td>28.4</td>
<td>11.6</td>
</tr>
<tr>
<td>175.8E</td>
<td>14.2</td>
<td>12.8</td>
<td>29.3</td>
<td>13.4</td>
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<tr>
<td>211.0E</td>
<td>13.9</td>
<td>13.5</td>
<td>28.9</td>
<td>13.5</td>
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*E = 0.5% emulsifier added

ANOVA

<table>
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<th>Source</th>
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<th>Sig. Level</th>
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<td>.521</td>
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<td>.053</td>
<td>.306</td>
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<td>C</td>
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<tr>
<td>Error</td>
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<td></td>
<td></td>
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</table>

A = TS  B = Emulsifier  C = HP
significantly with increasing homogenization pressure but there were no differences due to TS or to the presence of emulsifier.

The average equivalent spherical diameters de and the cumulative weight percentages above 10 micrometers are shown in Table 55 along with ANOVA of the results. There were no significant effects of the three processing variables on de, even though the weight percentage data showed a decrease with increasing homogenization pressure at both total solids levels studied.

Brookfield viscosities were also measured on the concentrates; the flow curves are drawn in Figures 82 and 83. As with the concentrates homogenized double stage, the flow is non-Newtonian. The results shown in Figure 82 for the samples homogenized single stage at 14% TS are somewhat anomalous in that the samples containing emulsifier fell on top of the other curves and the sample with emulsifier homogenized at 211.0 kg/cm² showed the lowest viscosity.
Table 55. Significance by ANOVA of Average Equivalent Spherical Diameter of WSDM Concentrates
Homogenized Single Stage

<table>
<thead>
<tr>
<th>Homogenization pressure kg/cm²</th>
<th>de &gt;8 micrometers</th>
<th>cumulative weight per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.0% TS Homogenized</td>
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<td>126.6</td>
<td>11.65</td>
<td>79.0</td>
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<tr>
<td>175.8</td>
<td>11.39</td>
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<td>10.83</td>
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<td>211.0E</td>
<td>10.33</td>
<td>53.3</td>
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*E = 0.5% emulsifier added

ANOVA

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<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Sig. Level</th>
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<td>.002</td>
<td>.0329</td>
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<td>B</td>
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<td>C</td>
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<td>.244</td>
<td>.122</td>
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<td>Error</td>
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</table>
A and D = 126.6 kg/cm²  
B and E = 175.8 kg/cm²  
C and F = 211.0 kg/cm²

Viscosity flow curves of WSDM concentrates homogenized single stage with (A, B, C) and without (D, E, F) emulsifier.

Figure 82
A and D = 126.6 kg/cm²  
B and E = 175.8 kg/cm²  
C and F = 211.0 kg/cm² 

Viscosity flow curves of WSDM concentrates homogenized single stage with (A, B, C) and without (D, E, F) emulsifier.

Figure 83
APPENDIX D

Coulter Counter Measurements of Soybean Flours

The average particle diameters of both full fat and defatted soybean flours were examined by Coulter Counter. In addition to \( d_e \), the values of \( d_1, d_2, d_3, d_4 \) and \( \overline{A} \) were computed. The results are shown in Table 56. It is immediately obvious that the average size distributions for full fat flour are a micrometer or more larger than those of defatted flour except for \( d_4 \). However, the full fat flour also contained many large particles that would not pass through the aperture and so were not counted; because of aperture blockage, fewer particles of full fat flour were counted as a result.

Log probability plots of the average volume particle distributions are shown in Figure 84. The curves show that the full fat flour had a greater weight percentage of particles above 10 micrometers; in addition, neither distribution was log normal although the data for full fat flour approached a straight line.

Specifications for WSDM (159) require that if full fat flour is to be used for product manufacture, 95% of the flour must pass through a U.S. Standard No. 100 sieve (149 micrometer openings). 95% of defatted flour must pass through a U.S. Standard No. 200 sieve (74 micrometer openings). Full fat flour is not milled as fine as the defatted flour because of the high fat content; therefore it was
Table 56. Average Particle Diameters of Soy Flours Dispersed in Water at 5.48% Total Solids (particles >8 micrometers)

<table>
<thead>
<tr>
<th></th>
<th>Full fat flour</th>
<th>Defatted flour</th>
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<tbody>
<tr>
<td>( \bar{d}_1 )</td>
<td>13.54</td>
<td>11.97</td>
</tr>
<tr>
<td>( \bar{d}_2 )</td>
<td>16.10</td>
<td>14.33</td>
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<tr>
<td>( \bar{d}_3 )</td>
<td>20.36</td>
<td>19.58</td>
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<tr>
<td>( \bar{d}_4 )</td>
<td>26.53</td>
<td>29.08</td>
</tr>
<tr>
<td>( \bar{D} )</td>
<td>14.77</td>
<td>13.10</td>
</tr>
<tr>
<td>( \text{de (D)} )</td>
<td>16.44</td>
<td>14.98</td>
</tr>
</tbody>
</table>
Log probability plots of particle size distributions of dispersed particles of soybean flours.

Figure 84
claimed (162) that products prepared with full fat flour should show greater phase separation upon reconstitution; for this reason, only defatted flour should be used for WSDM manufacture. The results for commercial samples did not support this claim. However, only defatted flour was used for experimental work because there are many more suppliers of defatted flour and it was believed that, since specifications permitted the use of any combination of flours and oil to achieve the final result, most suppliers would prepare WSDM with defatted flour.