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FUNCTIONAL COMPARISON OF SOY PROTEIN ISOLATE AND SODIUM
CASEINATE IN STABILIZED FAT MIXTURES

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Functional comparison of soy protein isolate

and sodium caseinate in stabilized

fat mixtures

by

Curtis Miles Amundson

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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LIST OF ABBREVIATIONS

C	Celsius
cm	centimeters
EC	Emulsion capacity
g	gram
G	Gravity
H+	Hydrogen ions
kcal	kilocalories
kg	kilograms
M	Molar
mg	milligrams
ml	milliliters
mm	millimeters
Na+	Sodium ions
NaCl	Sodium chloride
nm	nanometers
PER	Protein efficiency ratio
PSI	Per square inch
RPM	Revolutions per minute

INTRODUCTION

The stability of fat in a sausage product was most definitely a secondary concern when compared to the sensory qualities of the sausage for most of the history of sausage production. With the increasing economical pressure on sausage production, however, the use of relatively inexpensive fat trim types and the stabilization of that fat during thermal processing has become increasingly critical. The improvement of fat stability can be attempted in one of two primary ways. One way is by altering the process of sausage production and the other is by altering the formula of the sausage being produced.

One of the most successful processing methods of improving fat stability also defines a specific class of sausage products. This class is the finely chopped or comminuted sausage products often referred to as "emulsion" products. An emulsion is defined by Becher (1965) as a two phase system in which a discontinuous phase is distributed within a continuous phase. In meat emulsion products, such as frankfurters or bologna, the discontinuous phase is composed of fat, while protein and water constitute the continuous phase (Schut, 1976). In traditional meat emulsion theory, salt-soluble meat proteins, predominantly actin and myosin, form an interfacial film barrier around the lipid, stabilizing the lipid within the water environment (Visser, 1980).

The mechanical process of emulsion formation consists of chopping meat tissue for a specified period of time in the presence of salt. Unfortunately, if the mechanical action is insufficient for adequate protein extraction (Hamm, 1970), if overchopping of the meat batter takes place (Hansen, 1960), or if improper temperatures are reached during chopping (Walstra, 1984), fat stability may not be achieved.

A product's formula may be altered in several ways to improve the fat stability of a product. Obvious ingredients affecting emulsification of meat products include salt content and trim type. Lowering the quantity of fat trim relative to lean trim also obviously decreases the quantity of fat requiring stabilization. If, on the other hand, trim containing a high content of collagen is used, fat stability may be decreased (Acton et al., 1981).

Non-meat ingredients, other than salt, may be included in a sausage formula to promote fat stabilization. Several proteins, including cottonseed, sesame, rapeseed, soybean, and caseinate possess fat or water binding ability in sausage products (Vananuvat and Kinsella, 1975; Caldironi and Ockerman, 1982; Choi et al., 1983; Rivero de Padua, 1983).

One method of fat stabilization, developed relatively recently, promotes the process of fat stabilization through emulsification with a non-meat ingredient. This method involves the formation of a sodium caseinate, water and fat

batter (Brouwer et al., 1976; Jongsma, 1982; Visser, 1983a,b). This stabilized fat batter can then be added to a sausage product resulting in improvement of the sausage's fat stability.

Although popular in Europe, where stabilized fat has enabled sausage makers to increase the heat treatment their product undergoes, the use of stabilized fat has not had an impact in the United States. There are two key reasons for this. First of all, in the U.S. sausages are not thermally processed to particularly high temperatures. Secondly, and more importantly, in the U.S. the key mixture component, sodium caseinate, is only permitted in non-specific loaf products (U.S.D.A., 1973). As a result, the large quantity emulsion-type products manufactured in the U.S., including bologna and frankfurters, are not permitted by law to contain the caseinate stabilized fat mixtures.

The use of stabilized fat mixtures in meat products offers several potential advantages to U.S. meat processors. As previously mentioned, salt-soluble protein is used to stabilize fat in a meat emulsion. By adding already stabilized fat, lower quantities of salt-soluble protein are needed. This would enable a decrease in the relative quantity of salt-soluble protein in the system. The use of a stabilized fat source would also enable the fat and lean meat sources to be produced by preblending. Preblending, the chopping of lean

meat with salt and nitrite prior to sausage production, enables the determination of the precise proximate composition of the lean prior to product formation. Preblending can also improve the water and fat binding properties of the lean source (LaBudde and Selfridge, 1982). Preblending the fat souce would complement a lean trim preblend resulting in total product composition control. Stabilization of the fat meat may also enable the use of "novel" fat sources in meat products. Two such fat sources include cooked-off lard (currently a waste product) or vegetable oil (for a low saturated fat sausage).

From a strictly theoretical point of view, production of stabilized fat mixtures permits the study of non-meat protein in a system closely approximating the protein, water and fat composition of a food product.

The research presented in this dissertation had three primary goals. The first was to produce a soy protein stabilized fat mixture exhibiting stability equal to that seen in caseinate stabilized fat mixtures. The second goal was to compare a traditional method of measuring protein functionality (emulsion capacity) with the fat mixture method of measuring protein functionality. The final goal was to compare the effects of soy and caseinate fat mixtures when used as a fat source in sausage production. It was hoped that the research performed in order to achieve the project's goals might improve the understanding of factors involved in the stabilization of fat in conventional meat products.

REVIEW OF LITERATURE

Mechanism of Fat Stabilization

It makes very little difference in a sausage product how
the fat is stabilized, as long as it is indeed stabilized.
Whether a product's fat source is stabilized through a process
or a formula change, as discussed in the introduction, fat
stabilization must occur by one of three mechanisms. In the
first mechanism, the fat is stabilized in a highly viscous
matrix which simply inhibits movement of the fat. Flocculation
of the fat particles is thereby prevented. The key feature of
this mechanism is that the medium in which the fat is suspended
is an unorganized matrix. Another mechanism, called gelation,
prevents fat movement by trapping the fat particles in a three
dimensional matrix formed through specific protein to protein
interaction. In the third mechanism, emulsification, fat is
stabilized by dispersion. Depending upon the environment, the
rate of fat aggregation in the emulsion will vary. In an
emulsion, the stability of the emulsion is a function of the
frequency of collision of droplets in the discontinuous phase
material. In traditional meat emulsion theory, muscle proteins
improve the stability of the meat item by lessening interfacial
tension at the lipid and water interface. The following review
will examine the properties of isolated soy protein and sodium
caseinate. Since the majority of research on the fat
stabilization of meat products has been related to

emulsification, emulsification will be initially discussed.

Emulsification

Becher (1965) presented a more comprehensive definition of an emulsion than the one presented in the introduction. Becher described as emulsion as an "heterogeneous system, consisting of at least one immiscible liquid dispersed in another, in the form of droplets, whose diameters, in general, exceed 0.1 micron. Such systems possess a minimal stability which may be accentuated by such additives as surface active agents, finely divided solids, etc."

Emulsions in food products are usually one of two types, water-in-oil, such as margarine and butter, or oil-in-water, such as ice cream, salad dressing and cheese. In either case, small droplets of the discontinuous phase are dispersed in the continuous phase. The two phases in an emulsion, by definition, repulse each other and attract the like phase, resulting eventually, in aggregation of each phase into distinct layers (Petrowski, 1979; Friberg and El-Nokaly, 1983). In order to increase the stability of the emulsion, agents which exhibit attraction to both phases are often added to the emulsion. These agents function by aligning hydrophobic segments of their structure toward the lipid phase and hydrophillic segments into the water phase (Becher, 1965; Friberg and El-Nokaly, 1983). Numerous classes of emulsifying agents exist. One class consists of mono- and diglycerides.

Another consists of gums, and carbohydrate hydrocolloids, including alginates, carrageenans, and pectin. A third major class of emulsifiers, and the class of interest in this study, are proteins.

McWatters and Cherry (1981) decribe three processes involved in stabilizing emulsions. These are: (1) reduction of interfacial tension, (2) formation of a rigid interfacial film, and (3) accumulation of electrical charges. In the first process, lipid molecules in the interior of the lipid droplet orient their carbon chains in a symmetrical manner. In the water phase, polar orientation occurs. At the surface, where uncovered lipid contacts the water surface, neither orientation can interact. The emulsifying agent or surfactant orients non-polar areas (groups of non-polar amino acids in proteins) toward the lipid droplet. Polar areas (peptide groups consisting of charged amino acids) orient toward the water surface. Thus, the surfactant added forms a connection interacting with the polar and non-polar surfaces removing the surface tension which otherwise would exist between the lipid and aqueous phases. Many surface-acting agents tend to self-interact, filling in the entire interfacial area, forming an interfacial film (Stutz et al., 1973). If a protein is the surfactant, it is difficult to distinguish surface tension reduction from gelation stabilization. Film formation appears to be the primary method of maintaining emulsion stability

(Stutz et al., 1973). The reason for the stabilizing effect of accumulated electrostatic charges was explained by Petrowski (1979). He indicated that charges accumulated in lipid droplets tend to repulse like charges in other lipid droplets thus inhibiting lipid droplet flocculation.

Several methods are used to measure the ability of a surfactant to form emulsions. The most commonly used measure of surfactant activity is emulsion capacity (EC) (Kinsella, 1976). Emulsion capacity is defined as "the volume of oil that can be emulsified by a surfactant before inversion or collapse occurs." The method used to measure EC was originated by Swift et al. (1961). In this method, oil is added continuously to a protein/water dispersion. The total volume of oil added relative to protein at the point of breakage, then, is the EC. Originally, a visual decrease in viscosity was used to determine the breakpoint. Crenwelge et al. (1974) objectively measured breakpoint by the sudden drop of amperage in the blender due to the viscosity decrease of the solution at the breakpoint. Another objective measure, a drop in electrical conductivity at breakpoint caused by fat release, was developed by Webb et al. (1970). The most recent variation in endpoint determination is the color change when an emulsion formed with Oil-Red-O dyed oil breaks, releasing bright red oil to the surface (Marshall et al. 1975). Although variations of the Swift et al. (1961) analysis remain the most popular, other methods of measuring EC have been developed. Each method

is based on slightly different characteristics such as a lower
blender speed, (1750 RPM vs 13000 RPM), initial oil content
(Hegarty et al. 1963; Carpenter and Saffle, 1964) etc.

Several non-protein factors affect the amount of oil
emulsified using the EC test. The pH and ionic environment
of the protein solution affects the ability of the protein
to solubilize and be available for interface formation.
Kamat et al. (1978) indicated that near the isoelectric point,
a protein will have its greatest EC value because the protein
adsorption and elasticity at the oil/water interface is
greatest. Crenwelge et al. (1974) found, however, that pH 7.0
results in greater EC in meat proteins than the isoelectric
point.

Final temperature at emulsion breakpoint appears to have a
great effect on EC. Swift et al. (1961) and Carpenter and
Saffle (1964) found high negative correlations between
temperature rise and EC.

The effect of fat or oil addition rate on EC is not clear.
Swift et al. (1961) found a high correlation between increased
EC with increased rate of oil addition. They hypothesized that
the increase occurred because the protein layer formation was
nearly instantaneous and the greater the oil flow, the greater
the amount of oil emulsified prior to the onset of protein
denaturation. Carpenter and Saffle (1964), on the other hand,
found no difference when oil was added at rates from 0.21 to

1.56 ml oil per second and suggested that only overloading of the system or temperature differences due to excess duration of addition should affect the EC.

Increased mixing speed has been clearly demonstrated to decrease EC. Carpenter and Saffle (1964) found that a nearly perfect negative correlation existed between blender speed and EC in a range of blender speeds between 9000 and 21000 RPM when EC was tested using meat proteins.

The effect of oil or fat type on EC was studied by Christian and Saffle (1967). Little difference between different oils and fats was found with the exception of a decrease in EC when castor oil and linseed oil were used.

Methods other than EC exist for estimating protein functionality in emulsions. One such method is emulsion stability. Emulsion stability refers to the ability of an emulsion to undergo stress (usually heating) and remain unchanged (Hermansson, 1979). Several model system methods of measuring emulsion stability exist. The most frequently used stability measure consists of simply centrifuging a heated emulsion and then measuring the percent of emulsified layer remaining (Inklaar and Fortuin, 1969; Yasumatsu et al., 1972). Stability was measured by Carpenter and Saffle (1964) by cooking an oil/protein emulsion in a Paley bottle, adding hot water and centrifuging the bottle to measure fat release.

Another measure of emulsion stability was developed by Titus et al. (1968). An emulsion was formed and the water content of a 5 ml aliquot from the bottom of the emulsion was immediately measured. Twenty-four hours later another 5 ml aliquot was measured. An unstable emulsion showed increases in moisture in the bottom of the tube as the emulsion broke down and water and fat were released. The use of high speed blenders and a 30 minute delay were modifications suggested by Aoki et al. (1981). Trumbetas et al. (1976) used pulsed nuclear magnetic resonance to measure compositional changes in various layers of the emulsions.

A different approach to emulsion stability in dilute solutions was developed by Pearce and Kinsella (1978). They reasoned that the ability of a protein to emulsify is related to the amount of interfacial area coated by the protein. In a dilute solution, a relationship between interfacial area and turbidity exists. By measuring turbidity at 500 nm, an estimation of the protein's ability to form a layer at the lipid-water interface is obtained. This method seems to be gaining popularity currently (Hiratsuki et al., 1984; Nakamura et al., 1984).

One major problem exists with all of the previous analyses: they are all model system analyses. A model system is an evaluation of a functional property in a system that does not necessarily mimic the steps and ingredients of an actual food preparation (Pour-El, 1981). Model systems are used to

carefully examine a small number of factors with few interfering factors being present; they offer the advantages of simplicity, speed and relatively low cost. Unfortunately, their simplicity causes the results obtained to be of questionable value for estimating food protein functionality. Tsai et al. (1972) and Puski (1976) raised serious questions concerning the value of model system analysis. An example of this was seen by Saffle (1968) who indicated that model system emulsions containing 80% fat may be formed by less than 1% salt-soluble protein. In meat emulsions, however, a minimum of 9-10% total protein was required to emulsify a maximum of 35-40% fat.

Measurement of meat emulsion stability is done basically in one way. The emulsions are heated, and water and fat released during heating are collected. The most commonly used measure of emulsion stability was developed by Townsend et al. (1968). Emulsions, after being heated in plastic tubes were then decanted into 15 ml centrifuge tubes and centrifuged; the amounts of liquid, fat and gel-water, proteinaceous solids released were then measured. In the 18 years since its development, the original method has remained essentially unaltered. Earlier stability methodologies were developed by Meyer et al. (1964), Rongey (1965) and Saffle (1968). Of the three, the Rongey (1965) method still retains some popularity. The main feature of the Rongey method is the use of a glass centrifuge tube developed by Wierbicki et al. (1957) for

measurement of water holding capacity. The glass tube consists of a large top glass tube (2.5-3.8 cm diameter) connected to a small graduated bottom tube (1.0-1.3 cm diameter). The top and bottom tubes are separated from each other with a coarse, fritted disk. Emulsion is stuffed into the top tube and the assemblage is cooked. Following cooking, the tube is centrifuged and water, oil and solids released are collected in the smaller graduated tube and are directly measured.

Gelation

Another possible mechanism of fat stabilization by protein is the trapping or binding of fat in a protein gel matrix. According to Kinsella (1976), a protein gel is a "three dimensional matrix of intertwined, partially associated polypeptides, in which water is entrapped". A more specific description was offered by Schmidt et al. (1981) who defined gelation as "a protein aggregation phenomenon in which attractive and repulsive forces are so well balanced that a well-ordered tertiary network or matrix is formed". Two key features of both definitions are apparent. First, a three-dimensional structure is formed. Second, protein and water (in the case of a protein gel) are required. Ferry (1948) described two steps necessary to form a protein gel. First, the native protein must be denatured or dissociated by some mechanism to expose potential bonding locations. Second, reaggregation of the protein, forming inter- rather than intra-

molecular bonds, must occur.

The basic property which defines the structure and characteristics of the gel in question is the amount and type of crosslinking involved in the protein matrix formation. Four common types of bonding (covalent, hydrogen, hydrophobic and electrostatic bonding) are involved in gel formation (Schmidt et al., 1981). The four bonding types and their relative strengths are seen in Table 1.

In irreversible gels, covalent disulfide bonding functions to interconnect protein strands forming the basic gel structure. Non-covalent hydrogen, electrostatic and hydrophobic bonds function to stabilize the formed gel matrix. Covalent peptide bonding contributes little to any protein gel formation because chemicals needed to break peptide bonding will destroy the protein's primary structure. Irreversible gels are generally formed when a protein is treated with extreme heat, chemical or ionic treatment causing the breakage of intramolecular disulfide bonds. Once the severe treatment is removed, the protein refolds in a different manner than its native conformation. Random reassociation of sulfide residues results in an increased amount of intermolecular disulfide bonding (Schmidt et al., 1981).

When non-covalent hydrogen bonding predominates, gel reversibility upon heating is possible (Stainsby, 1977). Hydrogen bonding is less firmly structured than covalent

Table 1. The four common methods of protein bonding.

	Bonding Type	Strength	Method of Disruption
A.	Covalent bonding		
	Disulfide bonding	80-90 kcal/mole	disulfide reducing agents (including mercaptoethanol, sodium sulfite)
B.	Non-covalent bonding		
	1. Electrostatic interactions	10-20 kcal/mole	ionic agents
	2. Hydrophobic interactions	1-3 kcal/mole	urea
	3. Hydrogen bonding	2-10 kcal/mole	urea

bonding. This permits more space for water entrapment. As seen in Table 1, hydrogen bonds individually are relatively weak, but are so numerous as to significantly contribute to product stability. Addition of moderate heat can disrupt the hydrogen bonding, but removal of the heat allows reformation of the bonds. This is the basis of thermal reversibility.

Hydrophobic bonding can contribute to either reversible or irreversible gel formation. The key factor determining the formation of hydrophobic bonds is the initial denaturation of the protein's quaternary structure (Hermansson, 1978a). This denaturation exposes hydrophobic pockets allowing interaction upon renaturation.

Ionic bonding functions primarily to bind solvent to protein and thus may be critical to water stability, but not structural stability in gels (Chou and Morr, 1979).

Several factors affect gel formation. These include protein concentration, non-protein components, pH, ionic or reducing agents, and heat treatment. Specific factors affecting gel formation in isolated soy protein and sodium caseinate mixtures will be discussed later.

Measurement of Product Physical Structure

Following gel formation, using an appropriate combination of protein in solvent (8-20% protein) and gel forming

treatment (heating, ionic adjustment, etc.), or emulsion formation using an appropriate stabilizing agent, some measurement of structural formation can be made. Two classes of systems can be used to measure a protein gel's physical characteristics. One system is a model system which can be used to measure a mixture of two or three components. The other system is a food system containing the protein in question and several other components, simulating the actual conditions of protein usage.

Some "model system" type analyses of physical structure consist of measuring product structure by simply pushing some type of probe through the product. Several means of doing this have been developed. Rey and Labuza (1981) used an Instron probe to rupture the surface of the gel. Labuza and Busk (1979) studied the water absorbed from the product by capillary extrusion.

A more common method of estimating product structural formation is through the use of rotational viscometry. Hermansson (1975), Hermansson (1978b) and Shimada and Matsushita (1981a) utilized the Haake Rotoviscometer in measuring viscosity. According to Kinsella (1976), however, the most common method of measuring structure development is through the use of a Brookfield viscometer. Ehninger and Pratt

(1974) used a Brookfield viscometer with conventional disk-shaped spindles. The lack of flow properties exhibited by most products (including most gels), however, has dictated the use of T-spindles and a helipath stand attachments to the Brookfield viscometer (Circle et al., 1964; Catsimpoolas and Meyer, 1970; Catsimpoolas and Meyer, 1971a; Catsimpoolas and Meyer, 1971b; Fleming and Sosluski, 1975; Tung, 1978; Voutsinas et al., 1983). If the T-spindle is slowly lowered into the product, a circular pathway is cut through the gel with new product always being measured.

In the more complex "food system", the individual component contribution to the structure of the system is not always clear. The purpose of the system, however, is generally to estimate the final texture of the product. As with the model system analysis, several methods of measuring product structure exist.

Raw products, which have not yet stiffened during thermal processing, can be measured in the same manner as the model system, by measuring the product's propensity to exhibit flow behavior. As with gel measurements, one method to measure flow is through the use of rotational viscometry (Zayas, 1985). Most sausage batters, however, are not completely homogeneous mixtures and do not produce a consistent enough response during viscometry. An improvement over viscometry for sausage batters is a measure of the force needed to extrude the

product. Bourne (1978) described extrusion as a very effective method of estimating the structural quality of a material which can flow. Burge and Acton (1984) used fine or capillary extrusion of a product. Hermansson (1975) and Seman et al. (1980) measured extrusion using a coarse probe.

Solid products, such as most cooked meat products, present an entirely different problem. In cooked meat products, the ability to flow is severely restricted by the heat-set myosin gel. Several types of measurements are available, however, for solid meat products. Initially developed to measure meat tenderness, shear devices such as the single blade Warner-Bratzler and multiple blade Kramer shears have been used for sausage texture measurement (Lauck, 1975; Andersson, 1976; Fox et al., 1983; Sofos, 1983; Brady and Huneck, 1985). Compression of the product is a more common type of product structural analysis (Bourne, 1978; Seman et al., 1980). A method using double compression of the meat sample simulates initial chewing of the product. From the measured force curves, estimates of the product's hardness, cohesiveness, springiness, gumminess and chewiness can be derived. The original method was developed using the General Foods Texturometer (Brennan et al., 1970). More recently, however, an attachment to the Instron Universal Testing System has replaced the Texturometer. The Instron Universal Testing System's separation of the crosshead drive and compression cell from the calibration electronics

results in improved accuracy and precision of measurement over the earlier machine.

Structure and Function of Soy Protein and Sodium Caseinate

Both isolated soy protein and sodium caseinate are proteins (long chains of amino acids linked by amide bonds). In order to understand the mechanism of stabilization of the fat mixtures, some understanding of the structural and functional characteristics of each protein is necessary.

Soy protein structure

Soy proteins for food use are found in three principal forms (Wolf, 1977). The first, soy flour, contains 40-70% protein. The flours are formed by dehulling the whole soybeans or by dehulling and then defatting soybeans with hexane. By treating defatted flour with alcohol, acid or moist heat, a protein concentrate containing a minimum of 70% protein is formed. By extracting flour with dilute alkali at a pH of 7 to 9 followed by centrifugation and pH adjustment to pH 4.5, a protein curd is recovered. By combining the protein curd with sodium hydroxide and spray drying, a sodium soy proteinate or isolate is formed. A soy isolate must have a minimum protein content of 90%. A typical soy isolate has a proximate composition of 96% protein, 0.1% fat, 0.1% fiber, 3.5% ash, and 0.3% carbohydrate (Kinsella, 1979).

Fat stabilization requires the protein to function in either gelation or emulsification. To function as a gelling agent, several bond sites, unstable to denaturation but able to reassociate must exist. As an emulsifier, the soy protein must possess regions within the protein structure which express a hydrophilic nature and regions which express a hydrophobic nature.

The majority of soy proteins are globular in nature. Above the isoelectric point of pH 4.5, the proteins are soluble in water in the absence of salts. Ultracentrifugation of the water soluble globulins separates the proteins into four classes (Wolf and Briggs, 1959). One protein group is the 2S proteins which comprises 22% of the total proteins and consists of trypsin inhibitors, cytochrome C and other proteins (Wolf, 1972). The highest molecular weight protein fraction is the 15S protein which composes 11% of the total protein. The 15S protein may result from aggregation of the 11S protein fraction (Lis et al., 1966).

The two remaining protein fractions, the 7S and 11S proteins, constitute 68% of the total water soluble proteins. These proteins are called the soybean storage proteins. This indicates that these proteins are synthesized and produced within the seed, then stored until needed to supply amino acids

for the germinating seedling (Larkins, 1981). The 7S and 11S

proteins are considered to contribute most of the soy protein's

functionality through their ability to undergo association and

dissociation reactions (Wolf, 1970). The 7S fraction contains

hemagglutinins, lipoxygenase, and the 7S globulin called

β-conglycinin (Christopher et al., 1970). The 11S fraction is

a single protein called glycinin (Wolf, 1970).

Structural details of both the 7S and 11S globulins of soy

protein have been difficult to determine because of the

variability of breakdown products and the number of subunits

composing each protein. β-Conglycinin was initially believed

to be composed of 9 subunits (Koshiyama, 1970). Thanh and

Shibasaki (1978a) developed a different structural scheme based

on a β-conglycinin which is composed of six isomeric forms each

different because of their relative composition of α-, β-, and

α'- polypeptide subunits. The subunit structures of these

trimers are $\alpha'\beta 2(B1-)$, $\alpha\beta 2(B2-)$, $\alpha\alpha'\beta(B3-)$, $\alpha\alpha\beta (B4-)$, $\alpha\alpha'\alpha'(B$

$5-)$, and $\alpha\alpha\alpha(B6)$ (Thanh and Shibasaki, 1978a). The β-subunits

have lower contents of the amino acids glutamate, proline,

lysine and arginine and higher contents of the hydrophobic

amino acids valine, alanine, leucine, tyrosine, and

phenylalanine. Trimers B1 and B2 would possess greater

hydrophobicity than B3 and B4 which would in turn be more

hydrophobic than B5 and B6. The B1-B6 proteins possess a

circular structure, which at neutral pH and low salt
concentration associates or dimerizes to form a double circle,
9S, protein (Figure 1).

Although Wolf (1970) indicated that disulfide linkage was
a common property of 7S and 11S proteins, the effect of
disulfide bonding in the 7S protein appears to be, at best,
minimal. This is because only two cystine sites exist per
molecule (Wolf, 1972). Hoshi et al. (1982) indicated that
disulfide links did occur between α and α' subunits initiating
aggregation, but that β subunits did not form disulfide
linkages with α or α' subunits.

The 11S globulin, glycinin, has been easier than β-
conglycinin to characterize. As early as 1967, Catsimpoolas
et al. (1967) had hypothesized that 11S protein was composed of
at least 12 subunits. Only 8 subunits, four acidic (A1, A2,
A3, A4) and four basic (B1, B2, B3, B4), have been isolated
(Kitamura et al., 1976). The subunits are linked by disulfide
bonds in the following manner: A1 and A2 to B3, A3 to B1 and
B2, and A4 to B4. Badley et al. (1975) described the structure
of the 11S molecule as two similar monomers each consisting of
3 acidic and 3 basic subunits (Figure 2).

Chemically, the acidic subunits are larger in structure
having molecular weights of 37,000 to 42,000, while basic
subunits possess molecular weights of 19,000 to 20,000. In
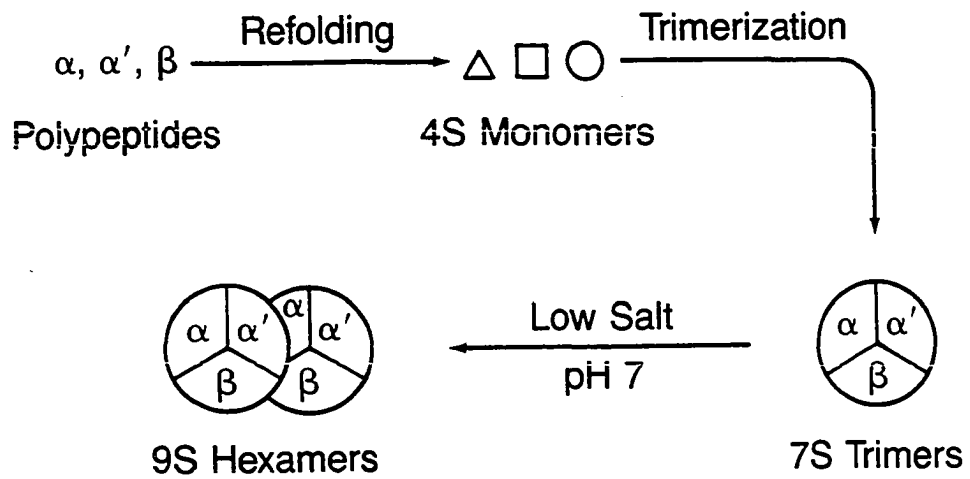
Figure 1. Formation of 7S and 9S proteins from alpha,
alpha prime and beta subunits (from Thanh and
Shibasaki, 1978a)
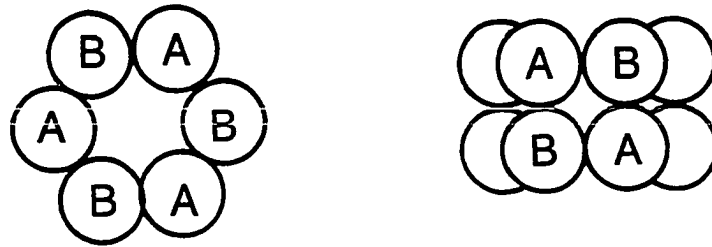
Figure 2.   Subunit arrangement in 11S soy protein
            (from Badley et al., 1975)

addition, acidic subunits contain relatively more aspartate and glutamate residues while basic subunits tend toward a more hydrophobic nature with larger quantities of leucine, valine and alanine (Moreira et al., 1979). Further research by Hoshi and Yamauchi (1983) more clearly defined the effect of disulfide bonds in 11S formation. Without disulfide reducing agents, the 11S globulin remained essentially unchanged with some A4 release. Addition of disulfide reducing agents resulted in separate banding on polyacrylamide gels of A4, A1-A3 and B1-B4 bands. It appears that at least six disulfide bonds attach basic and acidic subunits (Catsimpoolas et al., 1969; Badley et al., 1975). Other bonds involved in 11S formation are hydrophobic, electrostatic and hydrogen bonds (Wolf and Tamura, 1969).

Soy protein functionality

To be able to form a gel structure, a protein must have the ability for protein to protein interaction. The previous discussion of the 7S and 11S proteins indicated the presence of charged amino acids, hydrophobic amino acids and sulfur containing amino acids, all of which provide potential sites for protein to protein interaction.

To form a gel structure, native soy protein must first be denatured to expose bonding sites. Several specific protein denaturants exist including alcohol, which denatures hydrophobic areas; urea, which denatures hydrogen bonds; and

mercaptoethanol, cysteine, and sodium sulfite, which denature
disulfide bonds. General denaturing agents include alkali,
changes in pH or salt concentration and detergents. For food
use, however, the most important denaturant of soy protein is
heat.

When isolated soy protein is heated for 10 to 30 minutes
at 70-80°C, gelation occurs (Furukawa et al., 1979; German et
al., 1982). When soy is included in a food stuff, the
phenomenon of gelation is a critical contributor to textural
development. The steps which occur in soy gelation are shown
in figure 3. In step A, a sol phase consisting of a soy
protein dispersion in water is formed. In the sol phase, the
soy protein is hydrated by water, but denaturation is at a
minimum (Kinsella, 1976). Step B involves an irreversible heat
transformation of the sol to a progel state. The progel
formation is characterized by the denaturation of the native
soy intramolecular bonds. Denaturation is defined as a major
change from the native structure without alteration of the
amino acid structure (Tanford, 1968). With added heat, soy
intramolecular hydrogen, disulfide and hydrophobic bonds are
denatured (Shibasaki et al., 1969). This results in the
unfolding of the quaternary structure of soy protein and
release of the 2S, 7S, 11S, and 15S protein subunits together
with the exposure of internal subunit structure (Wolf, 1972).
The progel (labelled C) is an intermediate form identified by
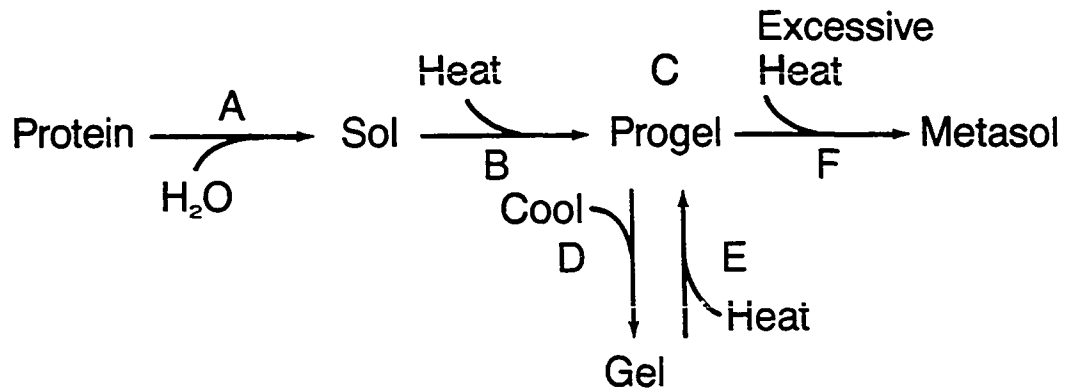
Figure 3. Heat gelation mechanism of soy protein isolate

an increase in viscosity of the sol prior to cooling and gel
formation (Circle et al., 1964; Catsimpoolas and Meyer, 1971b).
The increased viscosity characteristic of the progel is the
result of hydrophobic interactions between protein subunits.
These hydrophobic interactions are favored at high temperatures
(Scheraga, 1963).

In step D, cooling of the progel allows an orderly
reformation of hydrogen, ionic and covalent bonding patterns
resulting in gel formation. The relative importance of the
three types of bonding is unknown. Low concentrations of
disulfide bond reducing agents decrease the gel hardness of
heat-induced gels (Furukawa and Ohta, 1982). Hydrogen bonding,
promoted by low temperatures, would also be a potential method
of gel formation (Catsimpoolas and Meyer, 1971a). The gel to
progel reversibility due to heating and cooling shown in steps
D and E tends to support the value of hydrogen bonding as the
primary bonding source for soy gel formation (Tombs, 1969).
Prolonged excessive heat, however, results in metasol formation
(step F). The metasol formation was characterized by Wolf
(1970) as the aggregation of the 11S protein to form an 80-100S
aggregate which continues to increase in size until
precipitation occurs.

A further understanding of metasol formation is not
critical since metasol formation should not be possible during
fat mixture production. Gel formation is likely, however, and
to examine how heat induces gel formation in soy protein, the

first step is to determine how heat affects individual soy proteins. Prior to 1982, the only reported gelation study utilizing 7S protein was by Umeya et al. (1980). These authors measured an increase in hardness in 7S protein after heating and cooling. Measuring aromatic amino acids in 7S soy protein, Yamagishi et al. (1982) found few alterations in the quaternary structure after heating at 100°C. Phenylalanine residues were slightly more exposed, but not as much as when hydrogen bonds were disrupted. Tryptophan residues, on the other hand, appeared to locate even more into the interior of the molecule. Overall, heat did not have a major effect on the structure of isolated 7S protein.

At low protein concentrations (0.5%), 11S protein, when heated, became turbid and dissociated into acidic and basic subunits (Hashizume and Watanabe, 1979). At higher protein concentrations (5%), Mori et al. (1981) saw 11S aggregation followed by gelation. They concluded that protein concentration determined whether disaggregation or gelation occurred. Yamagishi et al. (1981) used a sulfhydryl blocker and still observed aggregation upon heating. When hydrogen and ionic bonding were inhibited, the aggregation seen was believed to occur by one of two mechanisms. Either hydrophobic bonding occurred or buried sulfhydryl groups were exposed which then became reactive. Nakamura et al. (1984) indicated that soluble aggregates of 11S protein, not precipitates, formed the

self-supporting gel.

In general, evidence indicates that little activity occurs in 7S proteins when heated, but 11S exhibits gelation behavior. Care must be taken, however, in relating individual protein behavior to soy gelation in general. Several interactions between subunits during heating are known. β-Subunits of the 7S protein and isolated 11S polypeptides were seen to spontaneously aggregate causing gelation at temperatures above 90°C through hydrophobic bonding (Wolf and Tamura, 1969). When complete 7S and 11S proteins were heated for 30 minutes at 100°C, however, no aggregation occurred (Damodaran and Kinsella, 1982; German et al., 1982). The lack of aggregation was attributed to a stabilizing effect of 7S protein which prevented 11S self-aggregation. The specific type of interaction was elucidated by Damodaran and Kinsella (1982) to be electrostatic in nature. They found that adjustment of the the ionic strength to 0.5 M NaCl, resulted in the breakage of the soluble 11S (basic subunit) and 7S complexes, permitting basic subunit aggregation. Yamagishi et al. (1983) found that disulfide bonding occurred between basic 11S subunits and the α-subunit of 7S protein. This interaction reportedly resulted in protein aggregation. Yamagishi et al. (1983) also found α,α', 7S interaction with the acidic subunit of 11S protein. Utsumi et al. (1982) confirmed the β-basic subunit interaction, but found no contribution of αα' subunits. They did find,

however, that the gelation mechanism of soy protein appeared to be a combination of basic to basic 11S subunit interaction via disulfide bonding and 11S basic subunit and β-7S subunit interaction via electrostatic bonding.

A gelation mechanism is well characterized during the heating of soy proteins. Schmidt et al. (1981) indicated that in systems where soy concentrates were utilized to stabilize fat, the mechanism of stabilization was by entrapment of fat particles followed by heat setting or gelation. One important feature seen in the previous study which is usually not seen in gelation studies was the presence of lipid material. The addition of fat results in a problem in interpreting results when the protein in question exhibits gelation and emulsification behavior. An early study by Catsimpoolas and Meyer (1971b) indicated that lower fatty acid chain length, increased lipid saturation, phospholipids and cholesterol tended to increase the soy/water/fat mixture viscosity. Kamat et al. (1978) indicated denaturation was required as an initial step to permit soy protein to lipid interaction. Shimada and Matsushita (1981b) also demonstrated an increase in product viscosity following the introduction of lipid into a water/soy protein mixture. Miura and Yamauchi (1983) explained this increase in viscosity in mixtures containing phospholipids. They felt a protein-phospholipid interaction increased the effective particle size resulting in increased intermolecular

entanglements. It is obvious, is that if a protein to lipid interaction results in the same textural increase as a protein to protein interaction, the measurement of that textural increase is a questionable method of measuring gelation.

Soy protein functionality is not necessarily limited to its ability to form a gel. It is also able to specifically interact with fat. The method of this interaction is unclear. The fat may be trapped in the protein network during protein gelation resulting in fat binding. Fat may also be emulsified with protein associating between the outer layer of fat droplets and the aqueous exterior. Regardless of the method of fat stabilization, however, fat stabilization occurring in studies incorporating soy protein is generally referred to as emulsification. Several methods of estimating emulsion characteristics have already been discussed.

Emulsion capacity and stability are the two tests most frequently used to estimate the ability of soy to emulsify fat. Using Supro 620, isolated soy protein, with an unadjusted pH of 6.88, Hutton and Campbell (1977) determined soy protein to have an emulsion capacity of 0.59 g of oil per g of protein on a dry weight basis. Added salt resulted in a general decrease in emulsified oil. The inhibitory effect of salt was greater at low temperature than at higher temperature (Ehninger and Pratt, 1974). Aoki et al. (1981) measured the emulsion stability of isolated soy protein as a function of pH and protein

solubility. They found that as protein solubility increased, stability also increased. Stability was shown to be minimal at a pH of 4.5 (isoelectric point), but rose substantially at pH 7.0. Results of Franzen and Kinsella (1975) and Volkert and Klein (1978) agreed with these findings, but Kamat et al. (1978) postulated that functional capability should be maximal at the isoelectric point. Flint and Johnson (1981) measuring oil-water interfacial film formation noted film formation from pH 1.0 to 6.5, but detection of film formation beyond a pH of 7.4 was impossible.

An elaborate examination of the functional capabilities of soy protein isolate during heating was performed by Voutsinas et al. (1983), who measured protein surface hydrophobicity using the fluorometric method of Kato and Nakai (1980). Protein hydrophobicity, protein solubility, fat binding capacity, emulsion stability, and emulsion activity index were compared. At a pH of 5.5, increasing the duration of heating was detrimental to protein solubility, emulsion stability and fat binding capacity when salt was included. Emulsion activity index remained unchanged and surface hydrophobicity increased. All characteristics measured except surface hydrophobicity increased to a great extent when the pH was increased from 5.5 to 7.2.

In order for classical emulsion formation to occur, the protein must absorb at the oil/water interface thus reducing

interfacial tension. Keshavarz and Nakai (1979) using soy protein isolate found that interfacial tension did decrease with increasing levels of protein hydrophobicity.

As with gelation, individual soy protein isolate subunits demonstrate differences in their ability to emulsify fat. The 7S globulin clearly exhibits greater emulsifying properties than 11S globulins (Saio and Watanabe, 1978; Aoki et al., 1981; Yamauchi et al., 1982). Aoki et al. (1981) noted that when individual soy proteins were isolated, the emulsifying properties of 7S globulin exhibited little sensitivity to heat while 11S globulin retained heat sensitivity. (This agrees well with the effects of heat on soy proteins discussed earlier.) Kamat et al. (1978) indicated that as was the case for gelation, the native protein had to be denatured before it would function properly as an emulsifier.

Caseinate structure

Milk proteins in the forms of caseins and caseinates are commonly used for the production of numerous food products including coffee whiteners, instant breakfasts, powdered toppings, etc. (Muller, 1971). For use in meat products, government regulations prohibit the use of caseinates in any but non-specific loaf-type products (U.S.D.A., 1973). The primary reason given for this regulation is the superior functional properties of caseinate. Caseinate usage would permit the use of either poor quality meat or the adulteration

of the meat product with high levels of water.

The manufacture of caseinate begins with pasteurization and cream removal from whole milk. The casein fraction of the milk is then precipitated by acidifying the skim milk to a pH of 4.5 (Kinsella, 1984). This is commonly accomplished using HCl, but may also result from acid production by fermentative bacteria. The acid precipitate is then neutralized to a pH of 6.7 by treatment with any one of a number of alkali compounds including NaOH, KOH, $Ca(OH)_2$, and NH_4OH (Muller, 1971). This treatment also enables the formerly insoluble casein protein to become a soluble caseinate. The resultant solution is then spray or roller dried to form a dry caseinate salt. The high viscosity of caseinate solutions requires an initial low total solids content to allow passage through the drier. This results in high drying costs and contributes to high product costs for caseinate salts. At this point, if the caseinate is to be used in a food product, sterilization occurs.

Whole caseinate consists of a number of subunit proteins. One group, called the α-s protein group, consists of a Ca++-sensitive protein group and constitutes 50-55% of the protein in casein (Bloomfield and Mead, 1974). β-Casein is less Ca++-sensitive and constitutes 30-35% of native casein (Morr, 1979a). A third group, the γ-caseins, accounts for 5% of the casein protein. (Several of the γ-caseins resemble portions of the β-casein chains and may actually be subunits of

β-casein.) The final casein subgroup, κ-casein, constitutes

15% of the total casein protein. κ-Caseins are not Ca++

sensitive and act as the protein component which allows the

assembly of the casein micelle when Ca++ is present (Bloomfield

and Mead, 1974).

Caseinate functionality

Caseinate demonstrates effective functionality as an

emulsifying agent. Measuring the emulsifying activity index of

sodium caseinate, Pearce and Kinsella (1978) rated caseinate as

a protein containing moderate emulsifying activity, lower than

succinylated yeast, but twice as functional as isolated soy

protein. Several factors affect this emulsification ability.

Inklaar and Fortuin (1969) dissolved 5 g of caseinate in 90 ml

of water, and after adding 50 ml of oil, measured several

emulsion characteristics. Although the nitrogen solubility of

caseinate was high (97.8%), the emulsion was unstable with

release of 54.5% of the oil used. An earlier study by Pearson

et al. (1965) examined the effect of salt type, ionic strength

and pH on emulsion capacity and stability. Potassium caseinate

proved to be an effective emulsifier. Mita et al. (1973) using

benzene in water stabilized by caseinate found a pH dependent

decrease in interfacial tension with the lowest tension at pH

6.0. In addition, the size the benzene globules formed was pH

dependent, with minimum size occurring at a pH of 7.0 or above.

Lower protein concentrations increased the relative emulsifying

capacity of the protein. This agreed with Sabharwal and Vakaleris (1972) who found maximum emulsion capacity at the lowest concentration of caseinate.

In food products, caseinates function to stabilize emulsions by stabilizing the oil-water interface in the classical manner described earlier (Phillips, 1981). Surface polypeptide chains also self-associate at the interface resulting in the formation of a protein film (Welsby et al., 1982). In addition, the entire product increases in viscosity, decreasing the ability of fat globules to coalesce (Welsby et al., 1982). When caseinate emulsion instability occurred, aqueous release was seen.

The molecular events involved in caseinate emulsification are well characterized. Caseinate consists of a non-uniform distribution of hydrophilic and hydrophobic residues (Bloomfield and Mead, 1974), which results in a ready capability to locate at the oil and water interface. In addition, sodium caseinate is highly soluble enabling distribution in the aqueous phase (Morr, 1979b).

The individual subunits of caseinate predispose the caseinate protein to a great deal of hydrophobic interaction. The major subunit of casein, α-s casein, consists of three predominantly hydrophobic regions located in amino acids 1-42, 90-113, and 132-199 (Mercier et al., 1973). The section 42-89 contains seven phosphorylated serine residues forming a small, but extremely hydrophilic, area (Brunner, 1977). The

hydrophobic regions are not buried when the α-s casein is found in the monomeric form. Once the casein micelle is disrupted, Ono et al. (1974a) estimated that 80% of the hydrophobic tyrosine and tryptophan residues are exposed to the solvent. Studies of the secondary structure by Ono et al. (1974b) indicated an unordered form predominated over α-helix and β-pleated sheet structure eight to one to one, respectively. This indicates that bonding in the α-s casein is predominantly through weak hydrophobic interaction (Jones, 1964). No disulfide bonding is possible in α-s casein because the necessary sulfide loci are lacking. The protein should thus easily be denatured, allowing maximum lipid interaction. Recently, the effect of specific segments of the α-s casein fraction in emulsification was investigated. Using limited hydrolysis, Shimizu et al. (1983) found that removal of the hydrophobic N-terminal segment (1-23 residues) reduced the ability of α-s casein to emulsify oil. Removal of the C-terminal hydrophobic segment, however, did not reduce α-s casein's ability to emulsify fat.

β-Casein contains a charged N-terminal area (amino acid residues 1-42) and a highly hydrophobic remainder (Ribadeau-Dumas et al., 1972). The hydrophobic area contains an even distribution of proline residues preventing α-helix structure. Arima et al. (1979) indicated that although β -casein possesses a nearly total random coil structure, it does

form a tight hydrophobically bound structure when heated.

The remaining primary casein is κ-casein. Unlike the α-s and β-caseins, κ-casein has the potential to form one disulfide bond between cysteine residues (Jolles et al., 1972). Swaisgood and Brunner (1963) suspected the formation of disulfide bridging on the basis of measurements of the dispersion of κ-casein in solution after addition of mercaptoethanol. Unlike α-s caseins, κ-casein contains only one phosphoryl serine residue. The primary hydrophilic area in κ-casein is a carbohydrate moiety attached to the 131 threonine amino acid residue (Brunner, 1977). As in the other caseins, a high content of distributed proline residues (11.8%) prevents the formation of a consistent ordered structure (Mercier et al., 1973).

In summary, the amphipathic nature of all major casein protein subunits together with a lack of ordered structure and covalent bonding sites should favor lipid-water interface formation. In addition, the lack of disulfide linkage sites should lessen the need for a great deal of protein denaturation in order to promote protein functionality.

Along with emulsification ability, casein possesses the ability to form a gel structure. The primary method of forming a gel with casein is to expose the casein to calcium concentrations of 0.4-0.6% (Welsby et al., 1982). Two possible mechanisms for this gelation have been suggested. Calcium may

interact covalently between phosphoserine residues on adjacent caseins or increased hydrophobic bonding may occur due to steric control by phosphorus-calcium molecular alignment.

Casein will also gel when exposed to the enzyme rennin. Once again, two theories for gelation exist. Tsugo and Yamauchi (1956) found breakage of α-s proteins into α-s1 and α-s2 fragments using rennin. This was believed to reduce casein solubility and result in the formation of a gel-precipitate. Wake (1959) proposed that κ-casein is attacked by rennin, breaking off the carbohydrate portion, forming para-κ-casein which prohibits proper solubilization resulting in gel-precipitate formation.

A critical aspect concerning gelation in casein protein is the lack of a thermal effect. The high heat stability of casein is generally attributed to its lack of ordered structure and to its lack of disulfide bond formation (McKenzie, 1967). Towler et al. (1981) did not find a decrease in viscosity after addition of disulfide reducing agents in casein solutions. Hayes et al. (1968) using calcium caseinate, described gel formation during heating. His gels formed at temperatures of 50-60°C., but reliquified upon cooling. In addition, in order to permit gelation, the addition of divalent cations was required. In general, gelation of casein proteins due to thermal effects is negligible in most food applications (Hermansson, 1975).

Use of Non-meat Proteins in Meat Products

Several non-meat food protein sources have been examined
for use in meat products. The reasons for this are numerous.
Some proteins such as soy and whey have previously been
incorporated in meat products (Gallimore, 1976), but are being
re-examined with a view to improve cost and supply factors.
Other proteins including plasma protein, wheat gluten, corn
gluten and cottonseed protein are being examined as new sources
of high quality proteins which are currently underutilized
(Siegel et al., 1979; Deshpande et al., 1983).

Initially added as filler proteins, non-meat proteins are
also added to contribute functional characteristics to meat
products (Rakosky, 1970). Functional characteristics most
commonly contributed by non-meat proteins include fat
emulsification, water and fat binding, and textural
improvement. Siegel et al. (1979) studied the ability of
wheat gluten, egg white, plasma, soy isolate, dried skim milk,
corn gluten and sodium caseinate to bind meat chunks by forming
gels. They found meat binding ability and gel forming
ability in these proteins, but through differing mechanisms.
Numerous studies have examined the emulsifying characteristics
of non-meat proteins. Caldironi and Ockerman (1982) studied
the emulsion characteristics of blood globin and plasma
proteins and found superior functionality in plasma protein,
but poor globin functionality. Ozimek and Poznanski (1981)

discovered an advantage in emulsion characteristics when textured milk protein was used to replace meat protein.

Non-meat proteins have had a long history as meat analogs. In 1866, J. Kellogg developed meat analogs made of nuts (Kinsella, 1983). The use of soy protein in analogs was not possible until Boyer (1954) developed a method to produce soy fibers which were suitable for human consumption. Meat analog sales in 1969 were estimated to be $10 million (Wolf and Cowan, 1975), and future markets for meat analogs appear assured as a result of nutritional concerns about meat products.

Soy protein has, however, been used to a greater extent for its functional properties as a meat product additive than as a meat analog. The first widespread application of soy protein in meat products was as a binder and extender in ground beef. The major impetus behind the use of soy in ground beef was a USDA ruling in February, 1971, permitting up to 30% soy extender in ground beef for the government school lunch program (Wolford, 1973). In March 1973, Red Owl Supermarkets introduced a 75% ground beef, 25% hydrated soy protein in 50 of its 400 retail stores (Horan, 1974). At a cost savings of 20 cents per pound, the extended beef was quickly accepted by the consumer and other supermarket chains. In early 1974, 30-40% of all supermarkets were carrying the soy-beef product (Gallimore, 1976). More than 30% of all ground beef was

purchased in the form of the soy-beef blend at its peak sales period. Gallimore (1976) indicated that the sales of the blend decreased to 21% of all ground beef by March 1974 and 10% by November 1975. Gallimore suggested that a decrease in beef prices lessened the appeal of the soy-beef blend, but recommended it as a method of stretching beef during periods of tight supply. At this time, the soy-beef blend is predominantly used in the school lunch program and the hospital/nursing home segment of the food service industry.

In ground beef products, when soy protein was used to replace meat proteins, several physical changes occurred in the characteristics of the resultant patties. McWatters (1977) saw improvement in water retention and protein contents using soy protein, but levels above 5% replacement adversely affected color and texture of the products. Andersson (1976) found a great decrease in patty firmness with 6, 12 or 24 % added isolated soy protein. It appears that the decrease in product texture was an important negative factor in the acceptance of soy extenders, but that the key factor was the "beany", or "green" off flavors found in soy protein blend products (Rackis et al., 1975).

A secondary use of soy protein, aside from simply to extend ground beef, is its use to contribute functionally to the meat product. This functional contribution includes emulsification and gelation improvement. As early as 1966, Rock et al. (1966)

demonstrated improved stability in marginally stable meat emulsions when soy protein isolate was added. Rakosky (1970) indicated that incorporation of soy isolate was a good method to improve appearance, texture and taste of comminuted meat products at a low cost. Several other authors have indicated improved texture resulted when soy protein was added to comminuted meat products (Hermansson, 1975; Sofos and Allen, 1977). But other reports indicate a decrease in textural firmness (Hermansson, 1975; Lauck, 1975; Comer and Dempster, 1981), flavor (Smith et al., 1973; Sofos, et al., 1977; Hand et al., 1983; Keeton et al., 1983) and color (Smith et al., 1973) when soy protein was added to comminuted meat products. Overall, the most obvious advantages to the use of soy protein in meat products appears to be its versatility and low cost, and the most obvious disadvantages are a decrease in firmness and a decrease in product flavor.

Several nutritional questions have arisen with increased addition of soy protein in the diet. Historically, the trypsin inhibitor activity of raw soybeans has been a concern. The trypsin inhibitor activity of several soy-added products was studied by Von Stratum and Rudrum (1979). They found no increase in trypsin inhibitor activity in soy-added sausages, minced meat or meat stews. A second potential nutritional problem with soy protein is lowered protein value or PER (protein efficiency ratio) values and amino acid scores seen

when soy is compared to animal protein. Cichon et al. (1980) found a decrease in PER in soy isolates or combinations of meat and soy isolates. The limiting amino acids are the sulfur containing amino acids. However, the lower sulfur amino acid content in soy protein is not considered a major problem in humans because of their relative lack of hair production (Young et al., 1979). The major concern about the use of soy in meat is the potential decrease in mineral absorption from the product due to the presence of phytic acid. Erdman (1979) indicated that the phytic acid in soy resulted in a lowered absorption of several minerals including zinc, iron, calcium, magnesium and phosphorous. Other authors including Welch and VanCampen (1975), Von Stratum and Rudrum (1979) and Young and Janghorbani (1981) indicated that most minerals in soy protein are readily available, so that combinations with meat should not alter mineral availability to any great extent.

It is obvious that non-meat proteins added to meat products result in assorted physical and functional changes. It is likely that the meat and non-meat proteins interact in some manner. Recently, a few researchers have begun to examine the molecular interactions between soy and meat proteins. Peng et al. (1982a) reported that 11S soy protein interacted with myosin when temperatures of 85 to 100°C were reached. The primary interaction found was between 11S and the myosin heavy

chain subunit. In a second study, Peng et al. (1982b) confirmed a myosin-11S interaction, but found little interaction between native muscle protein and 11S soy protein. Using electron microscopy, Haga and Ohashi (1984) found that the cold insoluble soy fraction (11S protein) formed networks with myosin B prior to heating. At temperatures of 70 to 100°C, a myosin gel supported by a surrounding soy gel appeared to form. Lin and Ito (1985) found that myosin B and soy protein resulted in a loss of extensibility compared to myosin B alone. They believed myosin B bound to actin in the myosin B complex simply by mixing with no heating. It appears that the potential for an interaction between muscle protein and 11S soy protein exists, but the conditions necessary for this interaction, and the effect of the interaction on texture, are not clear.

Unlike the use of the assorted soy proteins, the use of sodium caseinate in meat products has a much more limited history. The main reason for this is Federal Meat and Poultry Inspection regulations (U.S.D.A., 1973) which prohibits the use in the United States of caseinate in any product other than non-specific loaf products. Caseinate in Europe, is the primary non-meat protein used to stabilize fat emulsions. A relatively small number of studies has examined the functionality of sodium caseinate added directly to a meat mix. Caseinate when added to meat emulsions was shown by Comer and

Dempster (1981) to result in greater raw product stability than any of 12 other proteins including lean beef chuck and soy isolate. In the same study, however, a decrease in texture (gel breaking strength) was seen compared to all other proteins. Hermansson (1975) found no gelation possible using caseinate, and found that extrusion of meatballs produced with 4% caseinate required less force compared to isolated soy protein and whey protein concentrate. No increase in product strength occurred with heating of a caseinate solution. Siegel et al. (1979) and Terrell et al. (1982) were unable to achieve binding of meat pieces through the use of caseinate.

Stabilized Fat Mixtures

Although the formation of gels or measurable meat protein binding has not been demonstrated when heated, caseinate has found popularity in meat products as a fat stabilizer. One method of stabilizing fat using caseinate was developed in Holland by DMV, Inc. Methods for the production of stabilized fat emulsions using caseinate have been developed for several specific products including canned meats, liver sausages, minced meat, etc. (Hoogenkamp, 1979; Hoogenkamp, 1985).

Numerous factors affect the fat mixture made using caseinate. The specific type of fat, the location on the animal where the fat originated, the temperature of water used, the duration of chopping, the fat/protein/water ratio and the

manufacturing method affect stability (Brouwer et al., 1976; Visser, 1980; Jongsma, 1982; Visser, 1983a). Several of the factors have been well defined. Others, including manufacturing method and temperature of water used have been reasonably well characterized. Most of the data have, however, been developed by the industry.

Two non-industrial experiments dealing with the use of caseinate stabilized fat mixtures have recently been published. In one, Hand et al. (1983) formed a fat batter which was chunked and used in mortadella sausage. The caseinate fat mixture was found to have high weight loss, reportedly lost as encapsulated fat. In addition, sausage with this product added was rated much worse than a conventional product in cohesion, flavor and overall palatability. Zayas (1985) produced emulsified meat products using caseinate stabilized fat mixture. He determined that pre-emulsified fat made using caseinate contained smaller fat droplets which were much more stable to centrifugation than fat stabilized using soy isolate. Frankfurters produced using pre-emulsified fat exhibited greater viscosity and water holding capacity. In the finished product, yield was improved by 6-7%, but compression indicated a lower textural development. In most cases, sensory analysis of frankfurters was similar between products with or without fat mixtures. The only exception was tenderness, where products made with added emulsion were preferred.

Little data are available concerning the use of isolated soy protein in a stabilized fat mixture. Soy protein concentates in either 1/4/7 or 1/10/15 protein/water/fat ratio were produced by Schmidt et al. (1982). The 1/4/7 product developed much greater stability to pasteurization or sterilization than did product with lower levels or no soy protein concentrate. They concluded that soy protein concentrate surrounds tiny fat and moisture droplets, then stabilizes them as a result of heat induced gelation.

As described earlier with caseinate, Hand et al. (1983) produced mortadella sausage with fat mixtures stabilized using isolated soy protein. Less total weight loss was seen using soy protein than caseinate, but losses were predominantly in the form of free liquid oil. Products incorporating soy fat mixtures performed significantly worse in cohesion, flavor and overall palatability than did mortadella produced using pork backfat chunks.

Functionality of Meat Proteins

The primary interest in this study rests with the functionality of the two non-meat proteins, sodium caseinate and isolated soy protein. Addition of these proteins to a meat system, however, means that the functionality of the meat protein becomes intertwined with that of the non-meat proteins.

As mentioned in the introduction, emulsion-type sausages are noted for their improved fat stability. Several steps are

involved in the formation of the emulsion matrix of an emulsified meat product. First, the muscle protein is extracted in a salt and water solution. Maximum extraction was seen by Gillett et al. (1977) when a salt concentation of 9% was used. Hansen (1960) reported that after extraction, myosin and actomyosin concentrated at the fat globule surface. This lowered the interfacial tension at the oil/water interface resulting in improved emulsion stability.

While the myosin and actomyosin proteins are being extracted, the chopping process also serves to decrease fat particle size. A reduction in fat particle size both aids in the ease of distribution of the fat particles and decreases the relative protein coat to fat ratio. If overchopping takes place, the available protein will be extended over an excessive fat surface area resulting in a less stable protein coat and a less stable emulsion.

Microscopic examination of meat emulsions originally led researchers to the conclusion that fat particles were indeed covered by a protein coat (Helmer and Saffle, 1963; Borchert et al., 1967). Jones and Mandigo (1982) showed microscopic evidence of a protein coated fat globule. The stability of the protein layer was dependent upon chopping temperature. The warmer the chopping temperature, the greater the degree of rupture in the protein coating. Other authors, however, have criticized the emulsion theory of fat stabilization. Hamm

(1970) believed that fat is mechanically embedded in a protein matrix. Van Den Oord and Visser (1973) indicated that fat comminution typically performed is not sufficient for actual emulsion formation. Schut (1976) granted that a meat emulsion is not a "true" emulsion, but kinetically functioned in a manner similar to traditional emulsions. Recently, Katsaras and Stenzel (1984) and Schmidt et al. (1982) detected, using elecron microscopy, not only protein film coated fat globules, but also fat globules which contained no distinct protein coat.

Results of a limiated amount of research have indicated that the heavy meromyosin subfragment S-1 is the primary portion of myosin contiributing to fat emulsification (Kato and Nakai, 1980; Borjedo, 1983). Since surface hydrophobic regions are common in this region, minimal protein denaturation is required for film formation.

Meat provides one of the most obvious examples of protein gelation. Thermal gelation of muscle proteins induces the stiffening of the sausage matrix during processing. Schweid and Toledo (1981) determined that at 33-36° C, insolubilization or gelation of meat proteins begins. Hermansson (1986) indicated that with myosin, processing conditions may result in different types of gelation.

The mechanism of meat protein gelation has been studied extensively. Yasui and Ishioroshi (1980) determined myosin and actin to be the primary proteins involved in meat gelation.

Samejima et al. (1982), using purified myosin and actin together with troponin and tropomyosin, clearly demonstrated that the two regulatory proteins had no influence on gelation. The exact effects of actin and myosin are less clear. Samejima et al. (1969) indicated myosin was capable of self-association or gelation. Several authors have indicated that myosin is the key binding protein in meat sausages (Nakayama and Sato, 1971; Hegarty et al. 1963; Galluzzo and Regenstein, 1978).

Actin is known to self-associate forming linear gels (Oosawa et al., 1959; Maclean-Fletcher and Pollard, 1980). A study by Fukazawa et al. (1961) indicated that in a model system, actin would not self-associate. Actin has been demonstrated to potentiate the gel formation of myosin resulting in increased gel strength, but in order for actin to be effective, fibrous or F-actin is required. Yasui et al. (1982) indicated that maximum gel strength was formed in a system containing 80% myosin and 20% F-actin.

Samejima et al. (1985) presented evidence supporting a two phase gelation mechanism. In the first phase, the head region of the myosin molecule began gelling due to sulfhydryl bonding of the S-1 region with F-actin (Ishioroshi et al., 1980). This phase takes place at 20-25°C. At 50-70°C hydrophobic and hydrogen bonding due to denaturation and bonding contributions of the light meromyosin rod regions increase gel rigidity (Foegeding, 1983).

Until recent years, meat emulsion theory has predominated as the main method of fat stabilization in emulsified meat. Recently, however, emphasis has been shifting from emulsification to a "fat trapping" system. In this system, classical emulsification of a sort may contribute to stability of the product prior to thermal processing (Jones, 1984). During thermal processing, the aforementioned gelling of the meat proteins takes place, permanently trapping the fat pockets within the gel matrix (Ziegler and Acton, 1984; Comer et al., 1986).

EXPERIMENTAL PROCEDURE

Fat Mixture Production

Fat mixtures were produced by decreasing fat particle size to the point where the fat could be intimately associated with added protein and water. The key to the stability of the fat mixtures was the optimization of ingredient ratios and physical treatment of the mixture. To this end, the experimental procedure for part one of this experiment consisted of a series of minor ingredient and procedural changes designed to identify the optimum fat mixture process.

Mixtures of protein, water and backfat or oil were produced during this research. During the initial process of developing protein stabilized fat mixtures, the protein source (sodium caseinate or isolated soy protein) was mixed with water and pork backfat in the ratios presented. The protein/water/ fat numerical ratios were based on component weight.

Backfat used for the production of fat mixtures was obtained from the Iowa State University Meat Laboratory. Four and one tenth kg batches of fat mixture were produced using a Hobart model 84141D Laboratory bowl chopper which had two key modifications. First of all, an additional pair of blades was added to the original pair, increasing the chopping potential of the bowl chopper. Even more importantly, a steam heating ring around the bowl was added which permitted direct application of steam to the exterior surface of the bowl. In

combination with heated water, mixture temperatures of up to 60° C were possible. At lower temperatures, steam heating was used to maintain the specific temperatures required during the chopping cycle of the caseinate and soy stabilized fat mixture production (Tables 2 and 3).

A 1/8/8 (protein/water/fat) caseinate stabilized fat mixture was incorporated as a control with which to compare the soy stabilized fat mixture. The ingredients used to produce a 1/8/8 caseinate stabilized fat mixture are presented in Table 4. This ratio was derived from the work of various scientists at DMV (Brouwer et al., 1976; Jongsma, 1982; Visser, 1983a,b). The main experimental effort with caseinate was performed to optimize the production characteristics (chopping duration, chopping temperature) needed to produce the most stable fat mixture in the modified lab chopper. The combination of factors studied to produce caseinate stabilized fat mixtures is seen in Table 5.

Little information was available as a starting point for fat mixture formation using isolated soy protein. A 1/8/8 fat mixture using the same manufacturing methods used for the caseinate was used as the starting point. Table 6 presents the formulas used to produce 1/8/8, 1/5/5, 1/4/4, 1/5/4, 1/6/4, and the 1/4.5/4 fat mixtures manufactured during this study. In addition, Table 7 lists the production factors studied during the development of soy stabilized fat mixtures.

Table 2. Fat mixture production methods when protein is added to fat

Chopping Temperature (°C)	Water Temperature (°C)	Fat Treatment	Steam
26.6	26.6	21.1° C prechopped 1.0 minute	Initially to 26.6°C
37.8	54.4	21.1° C prechopped 1.0 minute	Initially to 37.8°C
43.3	77.1	21.1° C prechopped 1.0 minute	Initially to 43.3°C Intermittently to maintain
48.9	88.5	21.1°C prechopped 1.0 minute	Continuously to maintain 48.9°C
60.0	90.7	21.1°C prechopped 1.0 minute	Continuously to maintain 60.0°C

Table 3. Fat mixture production methods when soy protein is added to water

Chopping Temperature (°C)	Water Temperature (°C)	Fat Temperature (°C)	Steam
26.7	26.7	4.4	Initially to 26.6°C
37.8	60.0	4.4	Initially to 37.8°C
43.3	82.2	4.4	Continuously 43.3°C
48.9	90.6	4.4	Continuously to maintain 48.9°C
60.0	96.1	4.4	Continuously to maintain 60.0°C

Table 4. Ingredients used to produce 1/8/8 caseinate
          stabilized fat mixture

Ingredient	Weight (g)
Water	1814.4
Pork Backfat	1814.4
Sodium or Potassium Caseinate	226.8
Sodium Chloride	57.8
Sodium Nitrite	0.6

Table 5.   Factors examined in order to optimize caseinate
           stabilized fat mixture stability

Factor	Variables
Chopping Time	a. 5.0 minutes b. 7.5 minutes c. 10.0 minutes d. 12.5 minutes e. 15.0 minutes
Chopping Temperature	a. 26.6°C b. 37.8°C c. 43.3°C d. 48.9°C e. 60.0°C
Caseinate Type	a. EmHV-High Viscosity Sodium Caseinate b. PHV-Potassium Caseinate

Table 6. Ingredients used to produce soy stabilized
fat mixtures[a]

Fat Mixture	Water	Backfat	Protein	Sodium Nitrite
1/8/8	1814.4	1814.4	226.8	0.60
1/4/4	1814.4	1814.4	453.6	0.64
1/5/5	1814.4	1814.4	362.9	0.62
1/4.5/4	1921.1	1707.7	426.9	0.63
1/5/4	2016.0	1612.8	403.2	0.63
1/5.5/4	2100.9	1527.9	382.0	0.63
1/6/4	2177.3	1451.5	362.9	0.63

[a]Quantities of ingredients are expressed as grams.

Table 7.   Factors examined in order to optimize soy
           stabilized fat mixture stability

Factor	Variable
Chopping Time	a. 5.0 minutes b. 7.5 minutes c. 10.0 minutes d. 12.5 minutes e. 15.0 minutes
Chopping Temperature	a. 26.6°C b. 37.8°C c. 43.3°C d. 48.9°C e. 60.0°C
Soy Isolate Type	a. Purina 620T, 500E b. Grain Processing 902 c. Grain Processing 973
Addition Media	a. Chopped Backfat b. Water
Timing of Water Addition	a. Immediate addition to protein and fat mixture b. 60 second delay prior to addition
Protein/Water/Fat Ratio	a. 1/4/4 b. 1/4.5/4 c. 1/5/4 d. 1/5/5 e. 1/6/4 f. 1/7/4 g. 1/8/4

63

All caseinate and soy isolate fat mixtures which were
designated "protein added to fat" were produced using the
following manufacturing steps:

1. Chunked pork backfat was ground to 12.7 mm using a
   Biro model 9032 grinder.

2. Ground backfat was chopped in the Hobart modified
   model 84141D bowl chopper at 26.6°C until fat
   liquefaction (approximately two minutes).

3. The appropriate protein was added to the liquified fat
   during chopping.

4. Step 3 was followed either immediately or 60 seconds
   later by the addition of all but 100 g of the formula
   water. (The water temperature depended upon the
   factors under investigation.)

5. Following water addition, the mixture was chopped for
   a specified period of time.

6. Ninety seconds before the end of the chopping cycle,
   sodium nitrite dissolved in the remaining 100 g of
   water was added.

7. In caseinate stabilized fat mixtures, the required
   amount of sodium chloride was added 30 seconds prior
   to the end of the chopping cycle.

In some fat mixtures stabilized using isolated soy protein
which were designated as "protein added to water" the following
procedure was used:

1. The protein and water (less 100 g) were combined and
    chopped for two minutes.

2. Ground backfat was added and the mixture was chopped
    for the required chopping time.

3. A solution of sodium nitrite and the remaining 100 g
    of water was added 90 seconds prior to the completion
    of the chopping cycle.

After chopping, the fat mixtures were put in grey trans-
port tubs, covered, and held in a 5° C cooler. Laboratory
analysis was begun 18-24 hours after manufacture. Being
extremely homogeneous, the mixtures required no further
preparation prior to laboratory analysis.

Sausage Production Using Fat Mixtures

Caseinate control and soy isolate stabilized fat mixtures
were produced as described above for subsequent addition to
two of three product variations in which these fat mixtures
functioned as the fat source. In the third variation, 50% fat
pork trimmings were used as the fat source. Bologna and cotto
salami were selected as the test products for several reasons.
First, the effect of fat mixture on both an emulsified
and a coarse textured product could be compared. Second, a
relatively bland flavored product (bologna) could be compared
with a relatively spicy product (cotto salami). Third, both
products could be simultaneously processed in the smokehouse.

The formulas for the fat mixtures used in the sausage products were the 1/8/8 caseinate stabilized fat mixture (Table 4) and the 1/4.5/4 soy stabilized fat mixture (Table 6). One 4.9 kg fat mixture of each type was sufficient for a complete product replication. Three product replications were produced. The fat mixtures were produced 24 hours prior to sausage manufacture and held until manufacture in a -2.2°C cooler. Stabilized fat mixtures for use in cotto salami production were ground through a 3.12 mm grinder plate mounted in a Biro model 9032 grinder.

Fifty percent fat pork trimmings were recovered from pigs slaughtered at the Iowa State Meat Laboratory. The pork trimmings were selected from the same pigs supplying the backfat for fat mixture production. The pork trim was blast frozen at -30°C and maintained frozen until used.

The lean trim source, 90% lean beef trimmings, was obtained from a local meat purveyor. Lean trim was blast frozen at -30°C and maintained frozen for at least two weeks prior to use.

Twenty-four hours before product manufacture, all trim types were removed from the freezer and tempered to -2.2°C. The trim was then coarse ground through a 12.7 mm grinder plate mounted in a Biro model 9032 grinder. The lean and fat coarse ground trimmings were analyzed for fat content using the Katridg-Pak Anyl Ray.

The product formulations used for the bologna and cotto salami are shown in Tables 8 and 9. Bologna production began with the batching of ingredients for a 4.08 kg batch. The lean beef trim was placed in a Hobart laboraotry-sized chopper model 84181D modified as previously indicated. In addition to the lean trim, the salt and half of the water (added as ice) were added to the chopper. The trim, salt and ice were chopped together until the temperature of the mixture reached 7.2°C. The fat source (50% fat pork trim or fat mixture), nitrite, seasonings and remaining ice were then added into the chopper bowl. The entire mix was chopped to a final temperature of 14.4°C.

Cotto salami was produced in a batch size of 4.54 kg. All ingredients were placed in a Leland model 100DA mixer and mixed for five minutes. After mixing, the cotto salami mixture was fine ground using one pass through a 3.18 mm grinder plate in a Biro model 9032 grinder.

All meat batters (ground cotto salami and chopped bologna) were sampled for stability and texture analysis. The remaining batter was then stuffed into 50.8 mm by 609.6 mm fine prestuck fibrous casing using a hand operated sausage stuffer. After stuffing, the exterior surfaces of the casings were wiped and the sticks were individually weighed. The sausage sticks were labelled and hung on a smokehouse truck in a 2°C cooler. After all sausages of each replicate were completed, the sausages

Table 8.   Formula used to produce bologna[a]

Ingredient	Weight (g)
Beef Trim (90% lean)	2041.2
Pork Trim (50% lean) or Fat Mixture	2041.2
Ice	607.8
Sodium Chloride (without caseinate fat mix)	91.8
Sodium Chloride (with caseinate fat mix)	61.2
Kosher Bologna Seasoning (Heller's #531)	20.3
Sodium Erythorbate	2.25
Sodium Nitrite (without fat mix)	0.71
Sodium Nitrite (with fat mix)	0.40

[a] 9 lb meat block

Table 9.   Formula used to produce cotto salami[a]

Ingredient	Weight (g)
Beef Trim (90% Lean)	2270
Pork Trim (50% Lean) or Fat Mixture	2270
Ice	454
Dried Skim Milk	154.2
Sodium Chloride (without caseinate fat mix)	136.1
Sodium Chloride (with caseinate fat mix)	102.0
Ground Black Pepper	11.3
Cracked Black Pepper	8.5
Cardamon	5.7
Sodium Erythorbate	2.5
Sodium Nitrite (without fat mix)	0.71
Sodium Nitrite (with fat mix)	0.41

[a] 10 lb meat block

were placed in a Maurer-Sohne smokehouse for thermal processing. The smokehouse schedule used for the products is shown in Table 10. Immediately after processing, the surface of each sausage was toweled dry and the sausage was reweighed.

After cooling for 24 hours at 5 °C, the sausages were once again weighed, and samples were taken for chemical and textural analysis. The remaining product was vacuum packaged using a Multivac model AG800 dual chamber packaging machine and held at 2 °C until sensory and color analysis were performed.

Emulsion Capacity Measurement

Emulsion capacity (EC) was measured using the method of Swift et al. (1961) with several modifications. As in the Swift method, oil, water and protein were initially blended followed by oil addition. As opposed to the Swift method, corn oil rather than melted lard was used for the fat source. (Improved fat source consistency was anticipated by using corn oil rather than lard.) The protein source used was caseinate or soy isolate rather than extracted muscle proteins. Swift et al. (1961) utilized 1% salt solution in every case. (The salt was considered essential for muscle protein extraction and function.) In this experiment, distilled, deionized water or when indicated, 0.1 M or 1.0 M solutions of chemical grade NaCl in distilled, deionized water were used. The container used for the slurry formation was a container designed by Tantikarnjathep (1980) for emulsion capacity measurement.

Table 10.   Smokehouse cycle used for sausage processing

Time	Cycle	Temperature	Moisture 1	Impulse	Core Temperature
15 minutes	Reddening	54 °C	40 °C	---	---
1 hour	Hot Smoke I	60 °C	45 °C	85	---
Core	Hot Air Cook	70 °C	55 °C	85	58 °C
Core	Hot Air Cook	80 °C	65 °C	85	69 °C
10 minutes	Cold Shower	---	---	---	---

Ports into and out of the container were available for oil input and air displacement by the added oil. The container was attached to a Sorvall Omni-mixer, the speed of which was controlled using an electric rheostat. One hundred and fifty mg samples of isolated soy protein (Purina 620, GPC 902 or 973) or caseinate (DMV, EmHV or PHV) were suspended in 90 ml of distilled, deionized water, 0.1 M NaCl solution, 1.0 M NaCl solution or 30 ml of corn oil. (The medium in which the protein was dispersed will be denoted as water, 0.1 M NaCl, 1.0 M NaCl or oil.) Oil-Red-O dyed corn oil was homogenized into the protein dispersion using the Sorvall Omni-mixer at a rate of 33-36 ml per minute. Most emulsion capacity measurements were done with water and oil held at room temperature. For high temperature measurements, a 10-12°C temperature rise was achieved by using room temperature oil and 90°C water.

Emulsion capacity endpoint was determined using the Oil-Red-O dyed oil release method of Marshall et al. (1975). Red dyed corn oil was made by dissolving 0.3 g of Oil-Red-O dye in one liter of corn oil. This solution was magnetically stirred for 15 hours, then filtered through Whatman Number 1 filter paper using vacuum. All oil added during the emulsion capacity measurement included Oil-Red-O. Both an increase in color and a drop in viscosity occurred simultaneously at the endpoint.

Non-meat Proteins

Two types of caseinates were used in this study. High viscosity potassium caseinate (PHV) and high viscosity sodium caseinate (EmHV) were supplied by De Melkindustrie Veghel Inc., Holland. Soy protein isolate was supplied by Grain Processing Corp. of Muscatine, IA (Pro-Fam 902, 973) and Ralston-Purina of St. Louis, MO (500E, 620). Purina 500E was used in half of this research due to the inability to maintain a supply of 620. (Ralston-Purina's claim of functional equivalence between these proteins was confirmed by preliminary studies at Iowa State University. The difference between the proteins was the addition of titanium dioxide in the 620. Titanium dioxide lightens the color of the protein and serves as a tracer substance for 620 when used in meat products.)

Methods of Product Analysis

Proximate composition

Moisture content of all products was determined using the A.O.A.C. (1970) method with modification. The A.O.A.C. method uses 609.6 mm of Mercury vacuum with 95°C heat to remove the product's moisture. With caseinate stabilized fat mixture, this vacuum level resulted in the fat mixture expanding and creeping out of its container. By decreasing the vacuum to 10.2 mm of mercury and increasing the time of heating to 18-24 hours, constant dried sample weight was achieved. Fat content of the dried samples was determined using the petroleum ether

extraction method of A.O.A.C. (1970).

Chemical analysis

Rancidity in the fat mixtures and sausages was estimated using the thiobarbituric acid (TBA) method for malonaldehyde of Tarladgis et al. (1960). Results are reported as TBA number.

pH values of fat mixtures or sausages were determined using a Corning model 125 pH meter. Twenty g of sausage or fat mixture was homogenized for 30 seconds in 180 ml of distilled, deionized water using a Brinkman polytron Kinematica GmBH. An Orion 91-02 combination electrode standardized to pH 4 and pH 7 was lowered into the homogenate. The pH was read while the homogenate was continuously stirred. The pH of the emulsion capacity slurries was measured in a 50 ml aliquot of the slurry after emulsion breakdown. The method was similar to that used for sausages and fat mixtures, but no initial homogenation in water was necessary.

Functional analysis

Emulsion stability was determined by a hybrid method combining principles of Rongey (1965) and Townsend et al. (1968). Like the Rongey method, 30 g of fat mixture was stuffed into the top of a Wierbicki tube. Unlike the normal Rongey method, but like the Townsend method, incremental increases in waterbath temperature from 48.9°C to 80°C were used to heat the fat mixture in the tubes. The samples were heated to an internal temperature of 68.8°C. The samples were

then removed from the waterbath and allowed to cool for 4-5 minutes. The samples in the tubes were then centrifuged for 10 min at 1000 RPM in a Chicago Surgical & Electrical Co. model 61 centrifuge. Moisture, fat and solids released from the fat mixtures were measured directly from the graduated lower section of the Wierbicki tube. Stability measurements were also performed on the raw sausage product batters.

Product yields

Yields were calculated as follows:

Smokehouse Yield = Cooked product weight/Raw product
weight x 100

Final Product Yield = Cooked, cooled product weight/Raw
product weight x 100

Color analysis

Sausage product color was estimated using a Hunter Lab Labscan Spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The instrument was calibrated using a white standard plate (x = 1.018, Y = 1.019, Z = 1.012). Values for "L" (lightness), "a" (red-green) and "b" (blue-yellow) were measured using illuminant F (light from a cool white fluorescent source). A 50 mm port was used to allow maximum product surface exposure. All sausage products were sliced using a Hobart model 1712 slicer. Six slices were immediately vacuum packaged and stored in the dark until color was measured. The packaging material enabled color reflection off

of a smooth, flatter surface than would be presented on a cut surface. The average of the determinations for six sliced surfaces was used for product color estimation.

Structural analysis

Extrusion   Raw sausage and fat mixture samples were extruded using the method of Seman et al. (1980). Samples of warm fat mixture or raw sausage weighing 90 g were stuffed into 40 gram capacity, rigid, polystyrene containers having a diameter of 38 mm. After cooling at 2 °C for 15 hours, a 36 mm diameter plunger probe attached to a 250 kg load cell on an Instron Universal Testing Machine model 1120 was used to extrude the samples. A crosshead speed of 50 mm per minute and paper feed of 100 mm per minute were used to derive the extrusion curves. Results were reported as average kg of force necessary to extrude the sample. The independence of extrusion force to sample size (Bourne, 1974) eliminated the need to report the results on a per g basis.

Compression   Compression analysis of cooked sausage characteristics was performed using the double compression stroke method of Bourne (1968). This method utilized a curve derived from compressing a sample to 50% of its original height two consecutive times. From the curve, hardness (peak extrusion force), cohesiveness and elasticity were derived. Sixteen mm thick slices of each sausage product were sliced using a Hobart model 1712 slicer. The slices were compressed

to 8 mm thickness between a base plate and a 10.2 cm diameter
circular plate. The circular plate was attached to a 250 kg
load cell. Full scale load settings varied between product
types. A crosshead speed of 10 mm/min. and a paper feed of 50
mm/min. were used. All analyses were performed in triplicate.

Gelation determination   Gelation of soy and caseinate
proteins was measured using the method of Kinsella (1979).
Dispersions of one part of protein to ten parts of water were
made using distilled, deionized water at different
temperatures. The dispersions were mixed for 60 seconds using
a Sorvall Omni-mixer. Immediately after mixing, the
dispersions were decanted into 29 mm by 113 mm round bottom,
polycarbonate centrifuge tubes and placed in an ice water bath
for 5 minutes. The tubes were then centrifuged for 1 minute at
250X G in a Beckman model J21C Rotary Centrifuge. Tubes were
then equilibrated at 4°C. Structural formation of the
dispersions was measured in the tubes using a Brookfield HATD
rotoviscometer. A T-bar spindle (size F) and a helipath stand
were used to slowly lower the spindle through the dispersion.
The rotation speed of the spindle was 2.5 RPM. (It should be
noted that the samples exhibit a thixotropic flow behavior.
This means that different results may be obtained by using
viscometer speeds or a viscometer T-bar different from those
used.) Results are means from nine analyses.

Microscopic analysis

The sausage products and fat mixtures were frozen to -50 °C and cut into thin (16μm) sections using a Tissue Tech II microtome. Sections were placed on 25 mm by 75 mm microscope slides. Fat mixture sections were anchored on microscope slides with a subbing layer of albumin previously applied. (The fat mixtures were too fragile to remain intact during staining without using the albumin.) Non-subbed and subbed slides were dried and stained as follows:

1. Dry the slides under a lamp for 15 minutes.

2. Place slides in a propanediol solution for 30 seconds.

3. Stain the slides for protein, by placing the slides in Gill's hematoxylin solution for 30 seconds.

4. Rinse slides in moving water for 2 minutes.

5. Stain in Oil-Red-O solution for 10 minutes.

6. Rinse slides in water for 30 seconds.

7. Dry slides for 30 minutes.

The stained slides were examined suing a Bausch and Lomb Balplan microscope at 75 and 150 times magnification. Photographs were taken using a 35 mm camera attachment to the microscope and Ektachrome 400 speed slide film.

The structure of emulsion capacity mixtures was evaluated by placing two drops of the appropriate disperison on a microscope slide. The dispersions were made using Oil-Red-O dyed corn oil and received no further staining. (Preliminary

studies demonstrated protein clumping in these dispersions when protein stains were attempted.) As a result, oil was the only fraction whose location was visually identified with water and protein location being inferred.

Sensory analysis

Ten days following production, sausage products were evaluated for sensory attributes. Analysis of the bologna and cotto salami was performed by an untrained consumer panel. Panelists were asked to evaluate the products using a seven point facial hedonic scale for flavor, texture and overall acceptability (Figure 4).

After removal of the casing, the sausage products were sliced into 3 mm slices using a Hobart 1712 slicer. The slices were coded using random numbers and were refrigerated at 5°C until use. The samples were displayed at three specific stations each containing a specific fat source. All samples were displayed under red light during evaluation.

Statistical analysis

The Statistical Analysis Systems (Helwig and Council, 1979) was used for analysis of variance for all experimental data. Differences between means, when indicated, were examined using the Least Significant Difference test. All experiments were designed using randomized complete blocks. Fat mixture and sauasage production runs were replicated in triplicate except for the 1/8/8 soy stabilized fat mixture production

Sample Code _____		Flavor	Texture	Overall Accepta-bility
Like extremely				
Neutral				
Dislike extremely				
Comments:				

Figure 4. Visual seven point hedonic scale used to evaluate the finished sausage products

which was performed once. Emulsion capacity measurements were means of six replications.

RESULTS AND DISCUSSION

Fat Mixture Production

General characteristics

In attempting to optimize fat mixture stability, several production factors were examined. Even with assorted production factors, several characteristic similarities were shared by most of the fat mixtures produced. All caseinate mixtures were semifluid, retaining a definite, but slow, ability to flow. All soy mixtures were structurally solid possessing a rough, somewhat grainy texture.

The proximate composition of the fat mixtures proved to be remarkably consistent. The moisture content of both fat mixture types ranged from 47-53% of the product, while the fat content of the mixtures ranged from 37-42% of the product.

Despite the treatment used, the pH of the final mixture varied little within each protein type. Caseinate fat mixtures had a pH of approximately 6.7 pH units while soy fat mixtures averaged 7.0 to 7.2. Fat mixtures stabilized with a given protein tended to vary in pH to a greater degree between replications than between treatments within a replication. Two factors may have caused this. Either the fat source contributed to pH differences or the pH meter drifted in calibration with time. The latter was unlikely because the pH meter was calibrated just prior to each set of measurements. No attempt was made to control the pH of the fat mixtures.

Rather, the resultant pH of the "as is" mixture was measured. The possible influence of pH on the functionality of the two types of protein must not be ignored. It was felt, however, that commercial production of fat mixtures would become impractical if careful regulation of pH was a necessity.

Caseinate fat mixtures

It was very apparent that production of a stable soy-based fat mixture was the key initial step to this research. It was also apparent, however, that production of a stabilized fat mixture with no standard or control to compare it to would yield less information. Since production of a fat mixture stabilied by using caseinate had already been accomplished (Hoogenkamp, 1976; Jongsma, 1982; Visser, 1983a), it seemed a fitting control for this experiment. Visser (1983b) described several production characteristics for the manufacture of a fat mixture using several types of caseinate including EmHV, high viscosity sodium caseinate, and PHV, high viscosity potassium caseinate. A ratio of protein to water to fat of 1/7/7 or 1/8/8 was recommended. Use of 90°C water, a minimum chopping temperature of 45°C for mixtures containing pork fat, and a minimum chopping duration of five minutes was recommended when a high speed silent cutter is available. Since a low speed laboratory silent cutter and lower temperature water were used in this study, it was not possible to exactly duplicate Visser's procedures. As a result, a series of experiments was

performed to optimize the production methods for caseinate stabilized fat mixtures made using the low speed chopper.

The effect of temperature and caseinate type on product characteristics is shown in Table 11. None of the chemical composition factors shown in Table 11 discriminated between either the temperatures used or the proteins tested. With the use of higher chopping temperatures it was anticipated that the onset of rancidity may be hastened in the fat mixture. At one day after production, no differences in TBA values between temperatures were seen. One week later, the higher temperatures not only did not exhibit increased TBA values, the values were actually lower than their counterparts manufactured at lower temperatures. Granting that the fat mixtures contained 156 ppm Na^+-nitrite, TBA values indicated that no rancidity problem should exist if the fat mixture is used within a reasonable period of time. A comparison of the effects of temperature and protein type on the functional characteristics (stability and extrusion) of the fat mixtures is shown in Table 12. With EmHV, Townsend stability of the fat mixtures was not significantly different above 26.6° C. Potassium caseinate showed a maximum stability at 37.7°C and 48.9°C with somewhat lower stability at 26.6°C and 60°C. Above 26.6° C no differences between the two caseinate fat mixtures was seen in Instron extrusion levels.

Essentially, no difference existed between the two

Table 11. Effect of caseinate type and chopping temperature on chemical characteristics of fat mixtures[a]

Protein	Chopping Temperature (°C)	Moisture (%)	Fat[b] (%)	TBA[b] Day 1	TBA[b] Day 7	pH[b]
Sodium Caseinate	26.7	52.0[d]	36.8	0.033	0.191	6.70
	37.8	47.5[b]	39.6	0.036	0.246	6.69
	43.3	47.2[b]	41.9	0.045	0.200	6.73
	48.9	50.5[c,d]	39.1	0.023	0.086	6.74
	60.0	47.3[b]	42.1	0.044	0.068	6.72
Potassium Caseinate	26.7	51.2[d]	39.2	0.013	0.142	6.67
	37.8	48.3[b,c]	40.8	0.050	0.240	6.74
	43.3	49.8[b,c,d]	39.8	0.013	0.065	6.74
	48.9	50.9[c,d]	39.1	0.039	0.088	6.66
	60.0	50.5[c,d]	39.4	0.046	0.135	6.80

[a]n = 9 values per mean.

[b,c,d]Means within each column having different superscripts are significantly different (P<0.05).

Table 12. Effect of chopping temperature and caseinate type on emulsion stability and Instron extrusion [a]

Caseinate Type	Chopping Temperature ($^\circ$C)	Townsend Emulsion Stability (ml/30 g)	Instron Extrusion (kg)
Sodium Caseinate	26.6	1.5[b]	3.3[b]
	37.8	0.1[b]	4.8[d,e]
	43.3	0.0[b]	5.7[f]
	48.9	0.4[b]	4.3[c,d]
	60.0	1.3[b]	5.1[e]
Potassium Caseinate	26.6	0.5[b]	4.1[c]
	37.8	0.1[b]	4.8[d,e]
	43.3	0.0[b]	5.1[e]
	48.9	0.2[b]	4.7[d,e]
	60.0	0.1[b]	4.8[d,e]

[a]n = 9 values per mean.

[b,c,d,e,f]Means within each column having different superscripts are significantly different ($P<0.05$).

caseinates studied in stability, Instron extrusion, or any other characteristic measured (TBA, pH, proximate composition). As a result of these similarities, and since EmHV has a longer history of usage, EmHV was selected for futher experimentation.

This initial experiment was performed using the Townsend fat stabiltiy test commonly used for meat products. It quickly became apparent that this test would not be an adequate measure of stability for further experimentation. Caseinate stabilized fat mixtures could not be reliably decanted to measure moisture and oil losses. The mixtures would either completely flow out of the tube or they would not move in the tube. Although the flow properties of the mixture possessed interesting attributes, the erratic flow made consistent results impossible. In the original Townsend stability test (Townsend et al., 1968) raw meat emulsion stability was measured. During the heating process, meat fibers shrank away from the walls of the tube forming a smaller core surrounded by expelled fluid. The moisture and fat which separated during cooking was able to be decanted directly into a centrifuge tube. In caseinate fat mixtures, shrinkage and core formation did not occur, and moisture and fat would not always decant from the mixture. By switching to the procedure utilizing a Wierbicki tube (large diameter glass tube attached to a small diameter glass tube with the tubes separated by a coarse fritted disk) an improvement in consistency was seen. The new method was not

ideal. The flow properties of the caseinate fat mixtures enabled some of the mixtures to flow past the fritted disk into the smaller tube. During the centrifugation step, however, the oil and water separated easily.

Using the EmHV caseinate and the Rongey stability test, the temperature experiment was repeated. The results of this test (presented in Table 13) indicated that more residue was recovered using the Rongey stability measure. Although not significant at the $P<0.05$ level, trends in the stability of the caseinate stabilized fat mixture indicated (like the Townsend test), that the lowest (26.6 °C) and the highest (60° C) temperature mixes may be somewhat less stable than the other temperatures. The Instron extrusion values displayed much the same trend with temperature that stability measurements showed. The samples chopped at high and low temperatures had significantly lower Instron extrusion values than the samples made at the other three chopping temperatures.

The parallel between stability and Instron extrusion level may be significant when attempting to determine the mode of fat stabilization by caseinate. If classical emulsification theory is used to explain caseinate stabilization then the concurrent increase in extrusion value may be difficult to explain. This is because the emulsion theory per se, with film formation around fat globules being responsible for product stability, does not require interaction between the protein coating the

Table 13. Effect of temperature on the emulsion stability and Instron extrusion of sodium caseinate stabilized fat mixtures[a]

Chopping Temperature (°C)	Emulsion Stability (ml/30 g)	Instron Extrusion (kg)
26.6	1.4	4.7[b]
37.8	0.1	6.6[c]
43.3	0.3	6.8[c]
48.9	0.5	6.7[c]
60.0	1.0	5.2[b]

[a]n = 9 values per mean.

[b,c]Means within each column having different superscripts are significantly different ($P < 0.05$).

fat globules and the resultant viscosity increase.

The effect of chopping duration at 43.3°C is shown in Table 14. No difference in Instron extrusion values resulted from variations in chopping times. Total losses during Rongey stability measurements indicated a minimum of ten minutes of chopping in the lab chopper was required for a stable mixture. Although mixtures chopped for ten minutes were not significantly different in stability when compared to 12.5 or 15 minutes, stability tended to be slightly higher when a 12.5 minute chopping period was used. Visser (1983a) indicated that five minutes or less chopping time was required when a 45°C chopping temperature was used. At a chopping temperature of 50°C using the laboratory chopper (with 4 knives) at least twice the choping time was needed compared to a high speed chopper. Since the pork fat used in this research was preliquified, the chopping time must have been necessary to distribute the protein evenly in the fat mixture. It was apparent that the addition of two extra blades was not effective in increasing the small chopper effectiveness to the level of a high speed chopper.

The relationship between fat mixture textural formation and stability was not repeated in this experiment. Unlike the previous experiment, no increases in Instron extrusion values were seen in the more stable fat mixtures.

Table 14. Effect of chopping time on the emulsion stability
and Instron extrusion of sodium caseinate
stabilized fat mixtures[a]

Chopping Time (min)	Emulsion Stability (ml/30 g)	Instron Extrusion (kg)
5.0	5.1^c	4.3^b
7.5	5.1^c	3.7^b
10.0	3.0^{b,c}	4.8^b
12.5	1.1^b	4.7^b
15.0	0.8^b	4.8^b

[a]$n = 9$ values per mean.

[b,c]Means within each column having different
superscripts are significantly different ($P < 0.05$).

The experiments performed in attempting to determine the optimum production characteristics for the caseinate stabilized fat mixtures (as well as the soy isolate fat mixtures) were performed as a series of independent experiments with each one concentrating on a specific factor. As a result, the author acknowledges the possibility of undetected interactions (such as between chopping temperatures and chopping times). It must be remembered that the final fat mixture was tested for stability after manufacture, prior to use in a meat product. If acceptable stability was not evident, new fat mixtures would have been produced.

From the results presented, it was determined that the optimal caseinate stabilized fat mixture was produced in the laboratory bowl chopper in the following manner. Eight parts of fat were first liquified in the chopper. One part of caseinate was added to the fat paste followed immediately by 8 parts (less 100 gm) of 77°C water. After chopping the mixture for 11.0 minutes (maintaining 43.3°C using steam), 156 ppm of sodium nitrite (based on total mixture weight) dissolved in 100 gm of hot water was added. After 12 minutes of chopping, 1.5% sodium chloride was added. Chopping was concluded 12.5 minutes after the protein was added to the fat. Compared to the production recommendations of Visser, the only major change was an increase in chopping time.

Soy isolate fat mixtures

Unlike the caseinate stabilized fat mixture, fat mixtures stabilized using isolated soy protein have a very limited history. Only two studies have been reported in which an initial fat mixture stabilized using soy protein was formed. Schmidt et al. (1982) compared 1/10/15 and 1/4/7 soy isolate to water to fat ratios. Hand et al. (1983) produced relatively unstable (37.6% cook out) 1/4/4 soy stabilized fat mixtures.

In order to be consistent, a preliminary experiment was performed comparing three soy isolates and EmHV using 1/8/8 protein/water/fat ratios. The three proteins tested (GPC 902, GPC 973, and Ralston Purina 620) were recommended by the respective companies as soy isolates effective in fat emulsification. The stability results of this experiments are presented in Table 15. Only one replication of this experiment was performed because none of the soy products formed a stable fat mixture. It was interesting to note that the major component lost in the soy stabilized mixtures was fat, while only water was lost from the caseinate mixture. This was a trend which always occurred. Hand et al. (1983) found fat to be the primary component lost from soy stabilized mixtures, but they also found primarily fat lost from caseinate mixtures.

Since it was obvious that the soy isolate would not stabilize fat at a 1/8/8 ratio, an experiment was begun to compare two ratios recommended by Grain Processing Corp., Inc.

Table 15. Stability of soy isolate stabilized 1/8/8 fat mixtures[a,b]

| | Rongey Stability (ml/30 g) | | |
	Water	Oil	Total
Grain Processors 973	5.7	9.3	15.0
Grain Processors 902	3.9	10.6	14.5
Purina 620	3.6	10.4	14.0
EmHV Caseinate	1.7	0.0	1.7

[a]n = 3 values per mean.

[b]Only 1 replicate was performed.

The ratios were 1/5/5 and 1/4/4 protein/water/fat. The stability results are reported in Table 16. Once again higher fat than moisture loss was evident. These results indicated two things. First, the 1/4/4 protein to water to fat ratio outperformed the 1/5/5 ratio. Because maximum stability was considered a high priority goal, the 1/4/4 ratio was selected for further study. Second, when the losses over different ratios were compared, the GPC 973 protein outperformed the other two proteins. If, however, the 1/4/4 product was considered alone, the Purina 620 was slightly more stable. It is important to note that replication variability prevented any statistically sound method of choosing one protein over any other. Fat mixtures produced using Purina 620 were also found, by personal observation, to possess a more bland, less "beany" odor than fat mixtures produced using GPC 973. As a result of improved fat mixture aroma characteristics and slightly better, or at least not worse, stability ratings, Purina 620 was chosen for further experimentation.

At this point, the protein to be used (Purina 620) and the protein to water to fat ratio to be used (1/4/4) were selected. Several other factors, however, still remained to be optimized. One such factor was the optimum chopping temperature. During the previously reported experiments, fat was first chopped, then protein was added followed immediately by hot water. There was no reason to assume that soy protein added to fat

Table 16. Effect of type of isolated soy protein and protein/
water/fat ratio on emulsion stability[a]

Protein Type	Protein/Water/Fat Ratio	Emulsion Stability		
		ml Water	ml Fat	ml Total
620	1/4/4	0.1	0.7	0.8^b
	1/5/5	0.7	3.7	4.4^c
973	1/4/4	0.1	1.2	1.2^b
	1/5/5	0.9	1.1	2.1^b
902	1/4/4	0.2	1.3	1.5^b
	1/5/5	1.6	3.2	4.8^c

[a] n = 9 observations per mean.

[b,c] Means within each column having different superscripts
are significantly different ($P < 0.05$).

would make a more stable fat mixture than soy protein added to water followed by fat addition. An experiment was performed to examine chopping temperature and the medium (fat or water) to which the protein was added, followed 60 seconds later by addition of the remaining ingredient (water or fat). The results of this experiment are found in Table 17. Nothing unusual was seen in proximate moisture content or TBA numbers. The same could not be said, however, about Rongey emulsion stability.

All products in which protein was added to fat,followed by 60 seconds of chopping and then water addition, demonstrated poor product stability. This was unexpected because earlier mixtures had been stable when protein was added to fat followed immediately with water. Although the stability means were statistically different with different temperatures, no visible mixture diferences were evident. All mixtures resembled the product in Figure 5, in that they were creamy and smooth with no obvious external oil or fat puddles.

When the soy isolate was added to water followed 60 seconds later by ground fat, temperature sensitive results occurred. The effect of chopping temperature and soy protein addition to water is also shown in Table 17. Once again variation in moisture composition and TBA numbers are within acceptable limits. During production of these fat mixtures, differnces in stability were very obvious. At times, no stable

Table 17. Effect of addition order and chopping temperature on soy stabilized fat mixture characteristics

Addition Order	Chopping Temperature (°C)	Moisture (%)	TBA Day 7 (TBA Number)	Emulsion Stability (ml)	Instron Average (kg)
Protein Added to Fat	26.7	49.3^b	0.168^e	$5.90^{b,c}$	$4.4^{d,e}$
	37.8	49.6^b	$0.213^{d,e}$	$6.03^{b,c}$	4.2^e
	43.3	48.5^b	0.302^b	7.50^b	9.8^b
	48.9	47.2^b	$0.257^{c,d}$	$7.00^{b,c}$	11.0^b
	60.0	48.4^b	$0.279^{b,c}$	$6.17^{b,c}$	9.6^b
Protein Added to Water	26.7	48.2^b	0.195^e	$6.63^{b,c}$	$5.2^{d,e}$
	37.8	48.5^b	$0.221^{c,d}$	4.53^c	$6.9^{c,d}$
	43.3	51.9^c	0.179^e	4.70^c	$9.4^{b,c}$
	48.9	49.6^b	$0.263^{b,c}$	1.80^d	11.4^b
	60.0	47.4^b	$0.225^{c,d}$	1.93^d	11.4^b

[a] n = 9 observations per mean.

[b,c,d,e] Means within each column having different superscripts are significantly different (P< 0.05).
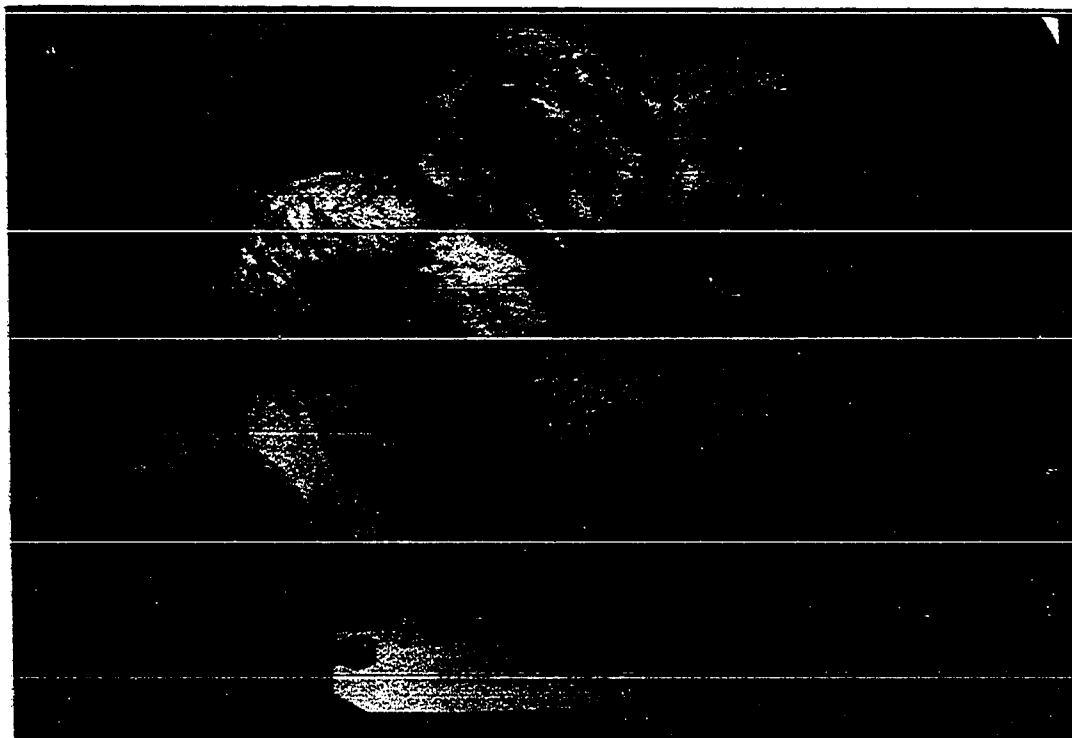
Figure 5.   Physical appearance of a formed soy stabilized fat
            mixture

structure was formed. Figure 6 shows what an unstable, unformed product looks like. This mixture should not be confused with the formed but unstable product described earlier, made by adding protein to chopped fat followed in 60 seconds with hot water. Although the stability results are similar in both cases, there was an obvious structural difference. Figure 5 presented the appearance of a protein added to water fat mixture which is stable. The smoother texture, uniform color and a lack of exposed oil make differences between a formed and an unformed mixture unmistakable. During the experiment, three replications of each of the five treatments (protein added to water) were made. When a chopping temperature of 26.6 °C was used, none of the mixtures were stable. At chopping temperatures of 37.8°C and 43.3°C, one of three fat mixtures were formed, and at 60°C all three formed. As seen in Table 17, Rongey stability differences bewteen formed and non-formed products were very obvious. It is interesting that the unformed mixtures made with protein added to water exhibited essentially the same stability as the protein added to fat mixtures. The stable mixtures produced using higher chopping temperatures were approaching the complete stability characteristics desired in a soy stabilized fat mixture.

In both protein added to fat and protein added to water mixtures, increases in Instron extrusion value generally
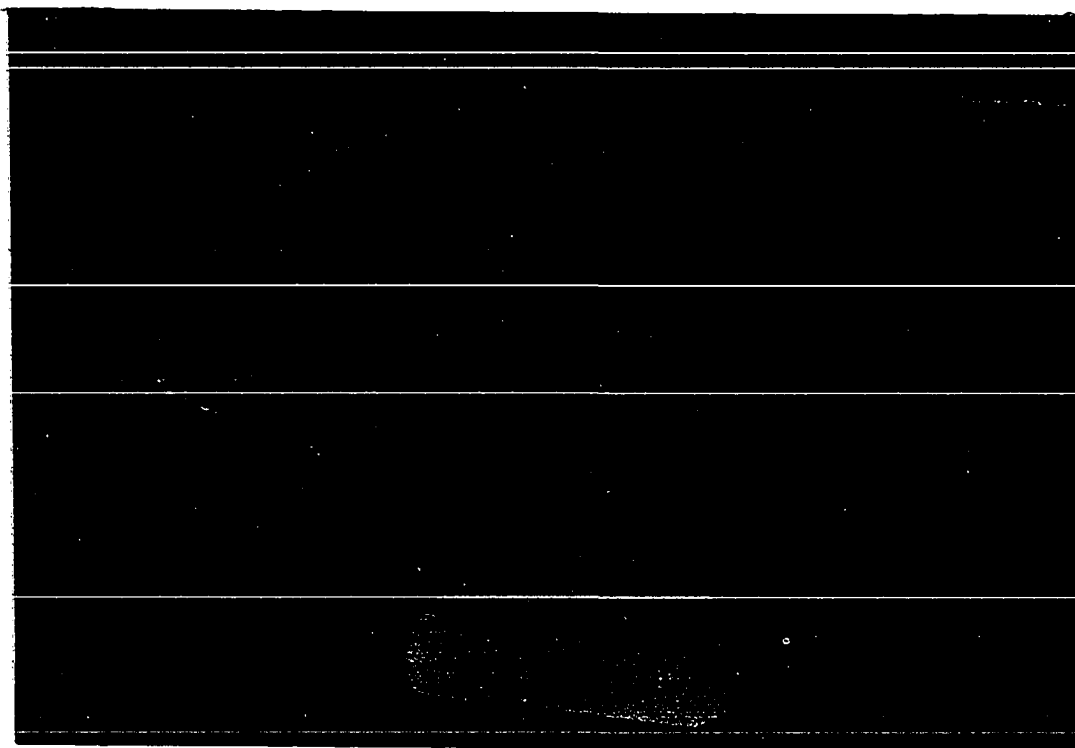
Figure 6. Physical appearance of an unstable soy stabilized
fat mixture (Note the presence of free lipid and
the coarse granular structure)

occurred with increases in chopping temperature (Table 17).
In the literature, an increase in physical texture of a soy
dispersion would be attributed to gelation of the soy isolate
(Catsimpoolas and Meyer, 1971a; Catsimpoolas and Meyer, 1971b;
Yasumatsu et al., 1972). Two factors in this experiment make
a gelation mechanism of stabilization questionable. First,
the presence of lipid introduces the possibility of some
potential structural formation resulting from emulsification.
Second, temperatures needed to permit soy gelation are usually
much higher than the chopping temperatures used in this study.

Previously, it was noted that increases in Instron
extrusion force often paralleled increased product stability.
In this experiment, when protein was added to water the trend
appears to continue (Table 17). When protein was added to fat,
however, no improvement in stability occurred with increased
Instron extrusion. This would indicate that although increased
product structural development may suggest improved stability,
solid support for this premise is lacking.

The experiment just reported, while informative, was a
step backward from the goal of product stability. Previously,
with immediate addition of hot water to protein and fat, good
stability characteristics resulted. In this experiment, a 60
second delay before water was added to soy stabilized fat
mixtures resulted in poor stability. Even worse, a 60 second
delay before fat was added to the protein-water mixture

resulted in either a very stable mixture or a mixture which would not form. As a result, another experiment was performed using only two chopping temperatures, 43.3°C and 48.9°C, but studying the delayed water or fat addition and immediate water addition. The Rongey stability and Instron extrusion results of this experiment are presented in Table 18. The mixture exhibiting the greatest stability was made by chopping the fat to a paste followed by protein addition and immediately following with the addition of hot water. As well as forming the most stable structure, the mixture also required the greatest Instron force for extrusion. Closer examination of the results from the third replication of this experiment showed what was perhaps the greatest revelation of this experiment.

During production of the protein added to water fat mixtures at 48.9°C, an unstable mixture containing free oil typical of some seen previously was produced. After sampling, water was added to the mixture in small increments. After an undetermined amount of additional water had been added, a completely stable mixture was suddenly formed. The Rongey stability values for the treatments in this replication are shown in Table 19. The desired fat mixture appeared to be possible by increasing water content somewhat. Samples of each fat mixture were thin sectioned, stained and examined under the light microscope. Figures 7 and 8 present the

Table 18. Effect of addition order and chopping temperature on soy stabilized fat mixture characteristics[a]

Addition Order	Chopping Temperature (°C)	Emulsion Stability (ml)	Instron Average (kg)
Addition of protein to water followed in 60 seconds with fat	43.3	2.77[b]	13.83[c]
	48.9	3.73[b]	14.92[c]
Addition of protein to fat followed in 60 seconds with water	43.3	7.60[c]	10.09[b]
	48.9	7.83[c]	10.97[b]
Addition of protein to fat followed immediately by water	43.3	4.40[b]	10.82[b]
	48.9	4.57[b]	9.47[b]

[a]n = 9 values per mean.

[b,c]Means within each column having different superscripts are significantly different (P<0.05)

Table 19. Effect of addition order and chopping temperature on emulsion stability of soy stabilized fat mixtures[a]

Addition Order	Chopping Temperature (°C)	Emulsion Stability		
		Water (ml)	Oil (ml)	Total (ml)
Addition of protein to fat followed	43.3	0.0	3.8	3.8[c]
immediately by water	48.9	0.0	5.6	5.6[c]
Addition of protein to fat followed in	43.3	0.0	7.8	7.8[d]
60 seconds with water	48.9	0.0	7.7	7.7[d]
Addition of protein to water followed in 60	43.3	0.0	0.4	0.4[b]
seconds with fat	48.9	0.0	4.2	4.2[c]
Addition of protein to water using additional water	48.9	0.0	0.0	0.0[b]

[a] n = 9 observations per mean.

[b,c,d] Means within each column having different superscripts are significantly different (P< 0.05).
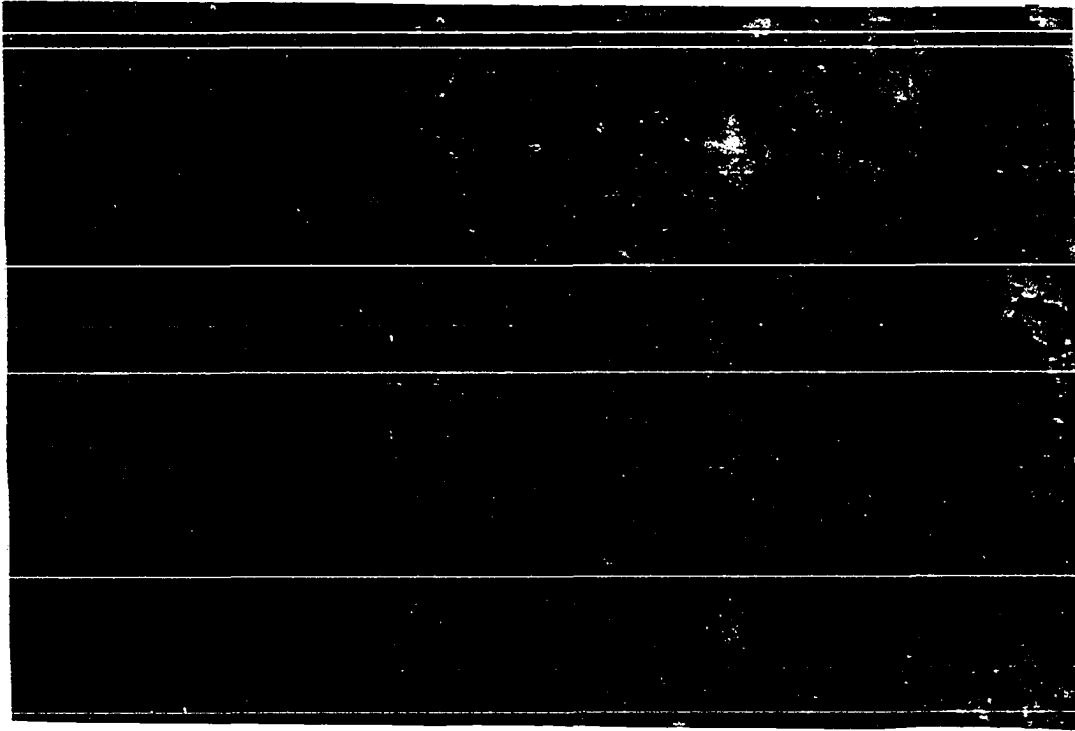
Figure 7. Light micrograph of a stained soy stabilized fat mixture when the protein and water were mixed prior to fat addition (Note the fine, filamentous protein structure (p) (100 X))
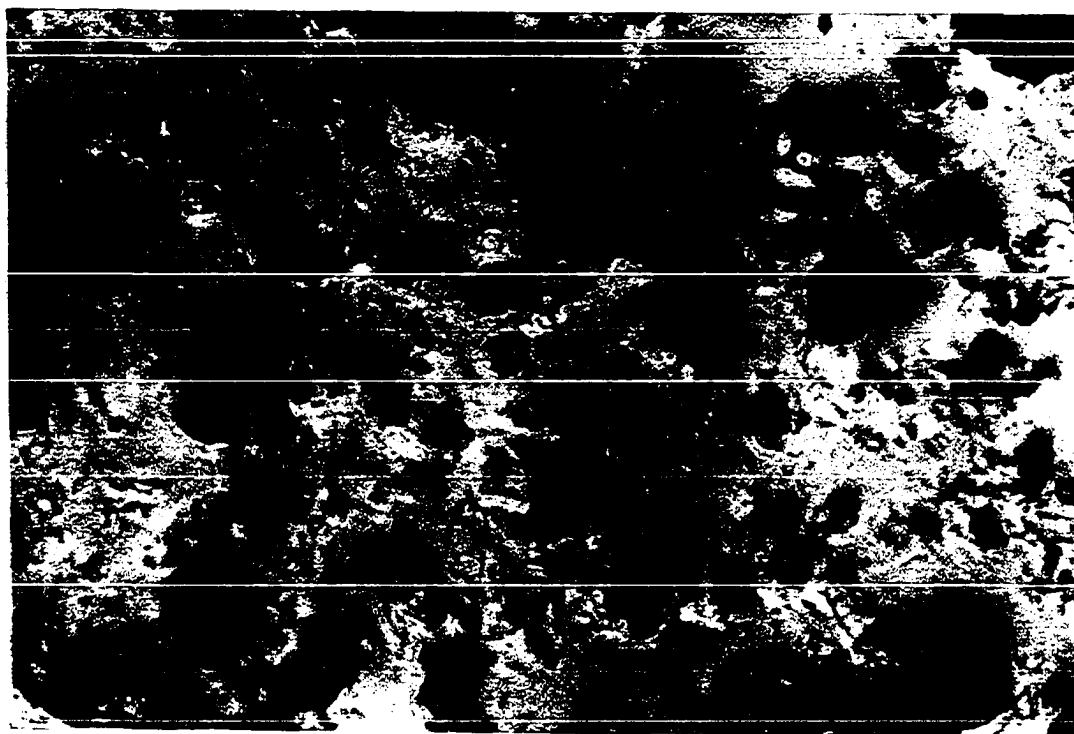
Figure 8.   Light micrograph of a stained soy stabilized fat
            mixture when the protein and fat were mixed prior
            water addition   (Note the dark clumps of unevenly
            distributed soy protein   (100 X))

stained sections. The product with added water (Figure 7) appeared to demonstrate an improved protein-water matrix resulting in retention of fat in a finer gel structure. When soy protein was added to fat followed 60 seconds later by hot water (Figure 8), a much different structure was noted. Large lipid stained areas were apparent with poorly distributed protein matrices being clearly visible.

At this point, it was apparent that increased water content in the fat mixture may be desirable. Information concerning the minimum additional water necessary to produce a stable soy-based fat mixture was required. It was important, in order to maintain a high fat content in the fat mixture, that the least additional water required be added. As the fat content of the mixture decreases, more and more of the mixture would be required to reach the final product target fat content. The final sausage product would, as a result, acquire sensory traits closer to the fat mixture.

The final experiment measured the characteristics of fat mixtures produced using soy protein to water to fat ratios varying from a 1/4/4 control to a 1/4.5/4, 1/5/4, and 1/6/4. The proximate composition together with the Rongey stability and Instron extrusion force values are presented in Table 20. The 1/4/4 fat mixture exhibited results comparable to previous experiments. Increased water addition resulted in predictable changes in proximate composition (Table 20). As little as a

Table 20. Effect of protein/water/fat ratio on soy stabilized fat mixture characteristics[a]

Protein/ Water/Fat Ratio	Moisture Content (%)	Fat Content (%)	Emulsion Stability (ml released)	Instron Average (kg)
1/4/4	45.7[b]	41.6[b]	2.97[b]	12.9[b,c]
1/4.5/4	47.4[c]	40.9[b]	0.03[c]	16.4[b]
1/5/4	50.9[d]	38.5[c]	0.03[c]	11.0[c]
1/5.5/4	52.7[e]	36.9[d]	0.03[c]	8.3[c,d]
1/6/4	54.4[f]	35.4[e]	0.00[c]	5.8[d]

[a] $n = 9$ observations per mean.

[b,c,d,e,f] Means within each column having different superscripts are significantly different ($P < 0.05$).

0.5 part increase in water content resulted in the formation of a completely stable fat mixture. No stability advantage in any further water addition was seen. The Instron extrusion values showed a peak in stiffness at a 1/4.5/4 ratio. The extrusion values then declined steadily with further increases in water content.

As a result of the previous experimentation, the optimum production characteristics of a soy stabilized fat mixture were determined. One part of Ralston-Purina 620 protein was combined with 4.5 parts water and 4 parts pork backfat. The water at 90°C was mixed with the protein for 60 seconds after which time ground fat was added. After chopping for 8.5 minutes 100 g water within which the nitrite was dissolved was added. Chopping was continued until ten minutes had elapsed since protein addition. Steam was continuously applied to the outside of the bowl to maintain 48.9°C temperature of the mixture.

Comparison of Caseinate and Soy Fat Mixtures

From the research performed, it was apparent that soy stabilized and caseinate stabilized fat mixtures possessed at least one stability feature in common. Both proteins were capable of trapping fat, in some manner, so that mild heating of the mixture did not result in fat or moisture release. Beyond this, however, more differences than similarities existed between the fat mixtures. By examining the

differences between the mixtures, an attempt was made to describe the mechanism of fat stabilization in both mixtures. The structures proposed are speculations fitting the data presented. No study of the fine structure of these fat mixtures was performed to confirm or deny proposed structures.

Stabilization of fat in systems such as the two described in this report may occur in one of three ways: through gelation which traps the fat in a three dimensional matrix, through emulsification of the fat particles, or through the trapping of the fat particles in a non-organized viscous matrix. Soy protein has long been noted for its propensity to gel when exposed to water and heat (Circle et al., 1964; Catsimpoolas and Meyer, 1970). Caseinate is generally reported not to gel in the presence of only water and heat (Hermansson, 1975; Terrell et al., 1982). In this experiment, it was questionable whether or not the water used was hot enough to promote soy gelation. In order to examine the possibility of soy or caseinate gelation under the conditions of this experiment, slurries of protein and water of the various temperatures used in this research for fat mixtures production were made. These slurries were measured and gelation determined using the method of Catsimpoolas and Meyer, (1970).

Density measurements were performed in this experiment to ensure against overestimation of structural formation due to

excessive compaction during the centrifuging step. Likewise, a major decrease in density would result in lower viscosity measurements due to the inability of the centrifugation step to remove air incorporated during protein slurry formation. In neither protein slurry were changes in density believed to materially affect the viscosity results.

In the case of soy protein isolate slurries (Table 21), a direct increase in viscosity occurred as the temperature of water used increased. Between 60°C and 70°C the greatest increase in viscosity occurred. During fat mixture production, the initial 60 second chopping of water and protein (when a protein added to water mixture was produced) was identical to conditions which promoted gelation in this study. Although a fat mixture contains other factors (i.e., fat and nitrite) it is safe to assume that the method used for final soy stabilized fat mixture production also possesses the conditions necessary to initiate gelation of the soy protein isolate.

The results of the caseinate slurry formation (Table 22) are equally clear, but completely opposite to those using soy isolate. As the temperature of the water used to produce the slurry increased, the viscosity of that slurry decreased. It is possible that another feature of the caseinate stabilized fat mixture formation may promote some protein to protein interaction (especially the 1.5% NaCl added); however, the increased water temperature did not appear to promote

Table 21. Effect of water temperature on the viscosity and density of isolated soy protein and water solutions[a]

Temperature ($^\circ$C)	Viscosity (10^6 CPS)	Density (g/ml)
10	1.3^b	0.92^b
20	1.2^b	$0.97^{b,c}$
30	$1.5^{b,c}$	$0.97^{b,c}$
40	$1.6^{c,d}$	0.99^c
50	$1.9^{d,e}$	$0.96^{b,c}$
60	2.1^e	$0.96^{b,c}$
70	2.6^f	$0.98^{b,c}$
80	2.9^g	$0.98^{b,c}$

[a] $n = 9$ observations per mean.

[b,c,d,e,f,g] Means within each column having different superscripts are significantly different ($P < 0.05$).

Table 22. Effect of water temperature on the viscosity and density of caseinate solutions[a]

Temperature (°C)	Viscosity (10^6 CPS)	Density (g/ml)
10	4.3[b,c]	1.06[b,c]
20	5.1[b]	1.07[b]
30	5.1[b]	1.08[b]
40	4.0[b,c]	1.07[b]
50	4.4[b,c]	1.06[b,c]
60	3.4[b]	0.95[c,d]
70	2.9[b]	0.92[d]
80	4.3[b,c]	1.01[b,c,d]

[a]n = 9 observations per mean.

[b,c,d]Means within each column having different superscripts are significantly different (P < 0.05).

gelation. It is interesting to note that although no increase in viscosity occurred, caseinate slurries were consistently much more viscous than soy protein slurries. No reason for this increased viscosity was determined.

By reexamining results presented in Table 21, Instron extrusion values tended to support the role of some gel structure formation in soy stabilized fat mixtures. When soy isolate was first added to water followed by fat, consistent increases in the force required to extrude the sample occurred with increases in the water temperature. The caseinate temperature results (Table 13) described an increase in extrusion force from 26.6°C to 45.4°C followed by essentially no change to 54.4°C and a **decrease in extrusion force** required to extrude a fat mixture prepared at 60.0°C.

In the results examined to this point, Rongey stability measurements presented no discernible stability changes attributable to temperature effects in caseinate fat mixtures. Soy mixtures which were exposed to greater temperatures, however, tended to also possess higher Rongey stability in samples where protein was added to water. The same result did not occur when protein was first added to fat.

It is possible that the type of fluid released from the fat mixture may give information concerning possible stability mechanisms. In caseinate stabilized fat mixtures, only water was recovered. This intuitively indicated that in our

experiment, caseinate was intimately associated with the lipid present, perhaps to a greater extent than it associated with water. The structure of caseinate described by Morr (1981), Ono et al. (1974b), Ribadeau-Dumas et al. (1972) and Mercier et al. (1973) certainly suggests that caseinate has a potential for lipid interaction. Protein to protein interaction, probably in a random fashion (coagulation), initiated by the ionic imbalance caused by the addition of 1.5% salt near the end of the chopping cycle probably traps the water not already inherent to the emulsion structure.

In soy stabilized fat mixtures, 99% or more of the fluid released during the Rongey stability test was lipid material. With soy stabilized fat mixtures, a lack of protein disperison together with low stability was found in fat mixtures in which the protein was first dispersed in lipid. In addition, oil was preferentially released from less stable mixtures. It was also apparent that additional water tended to improve the stability of the soy fat mixture. Water temperatures used appear to be high enough to promote protein gelation and mixtures with soy added to water which exhibit increased gelation potential also exhibit increased product stability. When all these factors are considered, the following mechanism of soy stabilized fat mixture formation can be hypothesized. Hot water and soy combine to begin the gelation process. The hot water denatures the soy protein promoting increased functionality. The hot

water denatures the soy protein promoting increased functionality. The water added is required to hydrate the protein and as a result is tightly bound. In fact, additional water (at least 4.5 parts) is required to hydrate sufficient protein to be assured of stabilizing four parts of lipid. As the unheated, ground fat is added, it is reduced in particle size and chopped into the soy-water progel matrix. The fat initiates cooling of the progel matrix and gel formation begins.

Protein Stabilization: Gelation or Emulsification

One of the major problems in studying protein interactions with lipid and water is the inability to differentiate between the possible stabilization methods, specifically gelation and emulsification. This is especially true in meat products. It is the opinion of the author that such a differentiation may never be possible. The reasons for this opinion are quite simple. Of the three mechanisms involved in emulsion stabilization, only two involve a protein stabilizer. These two mechanisms are decreasing interfacial tension and film formation (Wolf and Cowan, 1975; Schut, 1976; Morr, 1981). Of the two, film formation is believed to be the most important mechanism of emulsion stabilization (Tachibana and Inokuchi, 1953; Tachibana et al., 1957; Pearson and Alexander, 1968). If this is indeed true, then the three dimensional protein to protein interactions which form the film layer are primarily

responsible for the stabilization of an emulsion. Since a
three dimensional protein to protein interaction is also called
a gel, gelation forming the fat particle's film layer becomes
the key to protein stabilized emulsification. In addition,
those treatments used to break protein to protein bonds to
test for gelation would disrupt both a gel and an emulsion film
layer. If gelation stabilizes the protein film layer, then an
emulsion stabilized by protein is actually a gel.

Emulsion Capacity (EC) of Soy Isolate and Caseinate

The original emulsion capacity (EC) measurement, although
meant to permit a simplified measure of the functionality of
salt-soluble muscle protein, contained a number of specific
conditions (Swift et al., 1961). These conditions included the
use of melted lard as a fat source, salt (used to extract
salt-soluble protein), blender speed, speed of oil addition and
end-point determination. Many of these factors, as mentioned
in the literature review, have received various modifications
by several authors. Other factors, including the use of salt,
have remained essentially unchanged. One goal of this research
was to examine the impact on EC of certain factors found to
have significant impact on fat mixture production.

Effect of protein type

Just as the initial factor examined during the fat mixture
production was a comparison of several protein types, this was
the first factor examined using EC. The EC of several proteins

is seen in Table 23. In a no salt system, the isolated soy
proteins outperformed the caseinate proteins. Ralston Purina
620 soy isolate performed slightly better than the other two
soy isolates. Of the two caseinates, EmHV, sodium caseinate
had a greater EC than PHV, potassium caseinate. These results
appear to functionally justify the selection of both 620 as the
isolated soy representative and EmHV as the caseinate
representative for further study.

Effect of salt

Caseinate EC   Experiments were performed using
distilled, deionized water, and distilled, deionized water
containing either 0.1 M or 1.0 M NaCl. The effect of NaCl on
the EC of caseinate and soy isolate is seen in Table 24.
With the addition of NaCl at a 0.1 M level, an increase in the
EC of caseinate of 6 ml/100 mg of protein was seen. Two
reasons for this improvement are possible. First of all, the
ionic strength of the solution could promote unfolding of the
caseinate leading to exposure of interior hydrophobic groups.
The change in ionic strength of the solution could also alter
the type and amount of protein to protein interaction which
takes place, improving the film forming ability of the protein.

When 1.0 M NaCl solutions were used, a special condition
arose. Whether or not an actual decrease in EC occurred
depended upon the interpretation of endpoint. Most EC
experiments have declared an endpoint when a sudden decrease in

Table 23. Effect of protein type on emulsion capacity
and pH of emulsion capacity solutions[a]

Protein	Emulsion Capacity (ml/100 mg protein)	pH
Isolated Soy Protein (620, Purina)	50.8[e]	7.27[d]
Isolated Soy Protein (902, GPC)	47.8[d]	7.30[d]
Isolated Soy Protein (973, GPC)	47.4[d]	7.34[d]
Sodium Caseinate (EMHV, DMV)	44.7[c]	6.77[b]
Potassium Caseinate (PHV, DMV)	37.2[b]	6.91[c]

[a] $n = 18$ observations per mean.

[b,c,d,e] Means within each column having different superscripts are significantly different ($P < 0.05$).

Table 24. Effect of protein type and salt level on emulsion capacity[a]

Treatment	NaCl (M)		
	0.0	0.1	1.0
Isolated Soy Protein	56.0[f]	36.8[c]	33.7[b]
Sodium Caseinate	52.8[e]	58.5[f]	42.3[d]

[a] n = 18 observations per mean.

[b,c,d,e,f] Means having different superscripts are significantly different (P < 0.05).

viscosity occurred in the solution (Swift et al., 1961), when
stained oil was released from the EC solution (Marshall et al.,
1975), or when a drop in conductivity of the solution occurred
(Webb et al., 1970). The actual definition of EC is somewhat
different, however. EC is defined as the maximum amount of oil
which can be emulsified by a given amount of protein (Kinsella,
1976). The 1.0 M NaCl and caseinate solution lost the ability
to emulsify additional oil at a lower oil content than the
distilled water or 0.1 M NaCl and caseinate solution. It did
not, however, exhibit the drop in viscosity, loss of emulsion
matrix, or loss of electrical conductivity seen in other cases.
Although the inability of the protein to emulsify further oil
was apparent, it was probable that with the system being used,
no actual endpoint could have been reached. The large increase
in viscosity when 1.0 M NaCl was used indicated that an
increase in protein to protein interaction occured due to the
presence of salt. This increased protein to protein
interaction may also indicate the possible mechanism for the
improved EC which resulted with a 0.1 M NaCl solution.

Soy isolate EC   NaCl had a much more dramatic effect on
soy isolate EC than on caseinate EC (Table 24). When 0.1 M
NaCl solution was used the EC dropped 35% compared to EC in
distilled water alone. A slight additional drop in EC was
seen using 1.0 M NaCl solution. NaCl has been demonstrated to
have a negative effect on the functionality of soy isolate

(Hermansson and Akesson, 1975). This negative impact is believed to be the result of two effects of NaCl on the soy protein. The first effect is the maintenance of the native structure of the soy protein (Hermansson, 1972). Because of this inhibition of denaturation of soy protein, internal hydrophobically active areas and binding sites remain unexposed, limiting the protein's functional ability. NaCl also functions to prevent the renaturation of the protein previously denatured, decreasing the gel forming potential (Wolf, 1970).

The original EC test incorporated the use of 1.0 M NaCl to extract the salt-soluble proteins of muscle tissue. When conductivity is used as a method of endpoint determination, a solution including an electrolyte, such as 1.0 M NaCl, is required to conduct electricity. As seen in this study, the effect of all components in the system must be assessed. Had salt been retained in this study, soy EC functionality would have been significantly underestimated.

Removal of the NaCl from the remainder of the EC experiments was expected to underestimate the EC of caseinate. It should be remembered, however, that the goal of this portion of the study was to compare EC functionality with protein functionality in fat mixture formation. During fat mixture matrix formation, NaCl was not required for either type of stabilized fat mixture. (NaCl was added just prior to the end

of chopping in caseinate stabilized fat mixtures after the fat mixture matrix had been developed. The increase in viscosity seen after NaCl was added was necessary to ensure caseinate fat mixture stability.) In general, EC conditions should be used which reasonably simulate conditions which are expected in the actual product. If the protein is not designated for an actual product, however, an obligation exists to measure the EC of the protein under optimum conditions for that protein.

Effect of temperature on EC

The effect of temperature on EC is shown in Table 25. Since both proteins react to EC temperature increases in approximately the same manner, a discussion of the temperature trend does not need to be broken down by protein type. An experiment was performed using water at room temperature (22° C) or at 90°C. The room temperature water resulted in final EC temperatures of 31° C while the 90° C water resulted in final EC temperatures of 43° C. (The 43°C temperature was essentially the optimum chopping temperature during fat mixture production for caseinate and a minimum stable temperature for soy stabilized fat mixture production.) In every case, the EC measurements made using hot water were lower than the corresponding measurements made using the cooler water.

Other studies have indicated much the same temperature effect on EC. Swift et al. (1961) in the original EC measurement and Carpenter and Saffle (1964), both using meat

Table 25. Effect of protein type and temperature on
emulsion capacity[a]

Protein Type	Temperature	
	25° C	43° C
Isolated Soy Protein	44.2[c]	40.2[b]
Sodium Caseinate	56.2[d]	46.3[c]

[a] n = 18 observations per mean.

[b,c,d] Means having different superscripts are
significantly different (P < 0.05).

protein, found a significant decrease in EC with increasing EC solution temperature.

Effect of addition medium on EC

One other factor felt to have a potential effect on EC was the medium in which the protein was initially distributed. Most previous experimentation using muscle protein began with a ready-made water or salt-water solution in which the protein was dissolved (Carpenter and Saffle, 1964). This experiment did not require any specific solution in which to solubilize or extract the protein. In order to compare the initial dispersion effect, the protein of choice was dispersed by blending 150 mg of protein in 90 ml of either distilled, deionized water or 30 ml of corn oil. After 30 seconds of blending, either 90 ml of deionized water or 30 ml of corn oil (the opposite of the fluid first used) were added and EC measurement was begun. The effect of distributing the protein first in oil or first in water prior to blending is seen in Table 26.

This experiment was performed twice. The first experiment included the factors protein type, salt in the water, and temperature, as well as addition order. The dispersion medium actually had very little effect if the 1.0 M salt level is ignored. (The treatment combination of caseinate added to oil with 22° C, 1.0 M NaCl solution caused an assortment of significant interactions.) In the second experiment,

Table 26. Effect of addition medium and salt level on emulsion capacity[a]

Order of Addition	NaCl (M)		
	0.0	0.1	1.0
Dissolved in oil	54.3[e]	47.5[d]	35.4[b]
Dissolved in water	54.6[e]	47.9[d]	40.7[c]

[a]$n = 18$ observations per mean.

[b,c,d,e]Means having different superscripts are significantly different ($P < 0.05$).

dispersion medium was studied together with protein type as the only additional treatment. The results of this experiment (seen in Table 27) indicated a slight but significant difference in EC due to the dispersion medium with soy isolate, but no difference with caseinate.

Temperature and NaCl interaction

One specific combination of treatments (22°C water, protein addition to oil, caseinate protein, 1.0 M NaCl) resulted in a low EC estimate. The low EC measurement resulted in significant interaction effects being seen for several variables. The endpoint did not actually occur in this treatment; instead, a large increase in viscosity of the EC slurry was visually observed. This viscosity increase inhibited further oil addition to the slurry and resulted in EC termination. The increase in viscosity can be assumed to result from an increase in protein to protein interaction. The simple addition of lipid to a protein-water solution has been shown to increase the viscosity of solutions; however, the protein fraction of the EC test is more likely to be effected by the observed combined ionic and temperature effect.

EC slurry pH

It should be noted that several factors resulted in significant differences in end product pH in the endpoint protein/water/fat slurries. In all cases, protein type had a significant effect on pH with caseinate dispersions displaying

Table 27. Effect of protein type and addition order on emulsion capacity[a]

	Addition to Water	Addition to Oil
Isolated Soy Protein	32.7[b]	34.5[c]
Sodium Caseinate	40.0[d]	40.2[d]

[a] n = 18 observations per mean.

[b,c,d] Means having different superscripts are significantly different ($P < 0.05$).

lower pH values than soy dispersions (Table 28). The level of NaCl in the protein dispersion also played a significant role in end product pH. Increases in NaCl concentration resulted in consistently lower pH values (Table 28). This reflects the replacement of H+ ions on the protein by Na+ ions from the salt resulting in an increased H+ level in the solution.

Comparison of EC and Fat Mixture Production

Commonly, EC measurement or another simplified or "model" system is used to estimate the functionality of a specific protein as related to the protein's use in a food. In this study, fat mixture stabilization was directly compared to EC. Fat mixture production offers the advantage of comparing proteins in proportions similar to those which would occur in a food product, but without complicating factors such as other proteins etc. which would normally be present in a food. As a result, a fat mixture is nearly as simple a system as EC. By comparing the protein functionality as measured by EC and by fat mixture production, insights may be gained as to the relationship of the two types of methods. Table 29 displays the comparison of several factors which play a role in both EC and fat mixture production.

Several contradictions concerning the functional performance of the two proteins examined in EC and fat mixture production were apparent. The abilities of caseinate and soy isolate to stabilize fat in fat mixtures were clearly

Table 28. Effect of protein type and salt concentration on the pH of the emulsion capacity slurry[a]

	NaCl (M)		
	0.0	0.1	1.0
Isolated Soy Protein	7.23[b]	6.67[d]	6.32[e]
Sodium Caseinate	6.82[c]	6.59[d]	6.15[f]

[a]n = 9 values per mean.

[b,c,d,e]Means having different superscripts are significantly different (P < 0.05).

Table 29. Comparison of several factors involved in emulsion capacity and fat mixture production

Factor	Emulsion Capacity	Fat Mixture Production
Protein Type	Soy isolate had an equal or greater EC than sodium caseinate when no salt was present.	Caseinates and soy isolates produced stable mixtures. Soy isolates, however, required twice as much to provide the stable mixture.
Salt Content	Low (0.1 M NaCl) levels of salt increased EC of caseinate. Soy isolate EC was decreased with any salt addition.	NaCl was not used during fat mixture matrix formation. NaCl was required to ensure stability to the caseinate fat mixture, but was detrimental to a soy fat mixture.
Temperature	Increased temperatures resulted in decreased EC.	Temperature had little effect on caseinate fat mixture stability. Soy stabilized fat mixtures absolutely required mixture temperatures over $43.3°$ C develop fat mixture stability.
Addition Medium	With the exception of cold caseinate EC when 1.0 M NaCl was used, little difference was seen in EC when the protein was first distributed in either oil or water.	Soy isolate possessed poor stability when mixed in fat, was difficult to form when dispersed in water.

different. Caseinate stabilized fat mixtures were easily
formed at a protein to water to fat ratio of 1/8/8. Soy
isolate, on the other hand, was difficult to stabilize at
1/4/4 or 1/5/5 ratios and impossible to stabilize at the 1/8/8
ratio characteristic of caseinate. EC, on the other hand,
indicated that soy isolate was more effective than caseinate
in emulsifying oil.

The effect of temperature on EC and fat mixture production
was very different. The EC of both proteins was decreased by
increases in temperature. With caseinate stabilized fat
mixtures, temperature actually displayed little effect on
stability. At the lowest and highest temperatures used, some
slight reduction in stability was noted (Table 13).
Temperature had a large effect on soy isolate stabilized fat
mixtures (Table 17). Temperatures of at least 43.3° C
were critical to forming any fat mixture possessing reasonable
stability.

The medium (oil or water) in which the protein was
initially dispersed affected soy stabilzed fat mixture
production more than EC measurement. When soy isolate was
dispersed in fat 60 seconds prior to water addition, it was
impossible to produce a stable fat mixture. When first
dissolved in water, a stable mixture could be attained if other
factors, especially temperature, were optimized. (Caseinate
dispersion in water for fat mixture production was not

investigated.) The EC did not appear to be influenced by addition medium with either protein.

Salt was one factor which did not produce great differences between EC and fat mixtures measurements. In both EC measurement and fat mixture production, NaCl improved the functional properties of sodium caseinate. Only a modest improvement in EC of caseinate was seen with NaCl addition; however, preliminary experiments indicated that NaCl was critical for caseinate fat mixture stability. An interesting comparison can be made between NaCl incorporated into caseinate stabilized fat mixtures and the EC treatment using 1.0 M NaCl and 22°C water. Thirty seconds prior to the end of the fat mixture chopping cycle, 1.5% NaCl was added to the caseinate stabilized fat mixture. An immediate, visually observed increase in the viscosity of the fat mixture was noted. An increase in the viscosity of the EC slurry was also observed when 1.0 M NaCl solution at 22°C was used for the EC test treatment. NaCl has been demonstrated to increase the viscosity of a solution of caseinate in water (El-Negoumy, 1978), perhaps because of intermolecular crosslinking of phosphoserine and alanine promoted by NaCl. Morr (1979a) attributed the fat stabilizing properties of high viscosity caseinate to its ability to prevent aggregation of fat droplets simply because of the solution's viscosity. In the case of caseinate EC measurement, the viscosity of the slurry

is increased (with salt addition) to a point where the mixing action of the Omni-mixer is no longer sufficient to continue mixing the slurry.

In EC, NaCl was detrimental to soy protein functionality. Although the effect of NaCl was not tested in soy fat mixture production in this study, historically, the presence of salt has proven detrimental to soy isolate functionality (Wolf, 1970; Hermansson, 1972; Hermansson and Akesson, 1975).

It was apparent in this study that EC measurement was not effective in estimating the functionality of the proteins studied in relation to fat stabilization in fat mixtures. Assuming that EC measures the amount of oil the proteins emulsify, explanations for the reported differences in functionality can be formulated. The most obvious explanation is that emulsification was not the primary fat stabilizing mechanism in this particular experiment. In discussing soy isolate fat mixture stabilization, circumstantial evidence seemed to indicate that gelation, "fat trapping", may exert more influence in stabilizing fat than emulsification. In addition, the salt-induced stability of caseinate stabilized fat mixtures suggests that emulsification may not be the mechanism of caseinate fat mixture stabilization.

If, on the other hand, it is not assumed that EC measures the individual protein's ability to emulsify fat, then a closer examination of the EC method is required to determine what EC

is measuring. By producing slurries representing intermediate
forms of the EC measurement and examining them under the light
microscope, an attempt was made to determine the events which
take place during EC measurement.

In Figures 9 and 10, caseinate and soy EC slurries at
1/5/5 protein to water to fat ratios are shown. Under the
rather low magnification used in this experiment, no
structural details were apparent. The same was essentially
true with caseinate and soy EC slurries at 1/10/10 ratios
(Figure 11 and 12). At a ratio of 1/25/25 caseinate EC
slurries (Figure 13) and 1/50/50 soy EC slurries (Figure 14)
some distinct structures were becoming apparent.

At a ratio of 1/50/50 the caseinate EC slurry was shown
to develop several sizes of cells (Figure 15). An EC cell, in
this report, is a discrete packet of lipid and water bordered
by a protein film. Ratios of 1/100/100 in caseinate EC
slurries (Figure 16) produced a more defined structure as well
as an increase in the number and size of cells. Individual
droplets of stained lipid were seen at this ratio. In the EC
slurry seen at a caseinate ratio of 1/150/150 (Figure 17), the
presence of several very large cells with fewer small
segregated cells were apparent. At a ratio of 1/200/200
caseinate, EC breakdown had occurred. In general, with
caseinate EC slurries, as fat and water levels were increased,
larger and larger cells were formed. At the EC breakdown
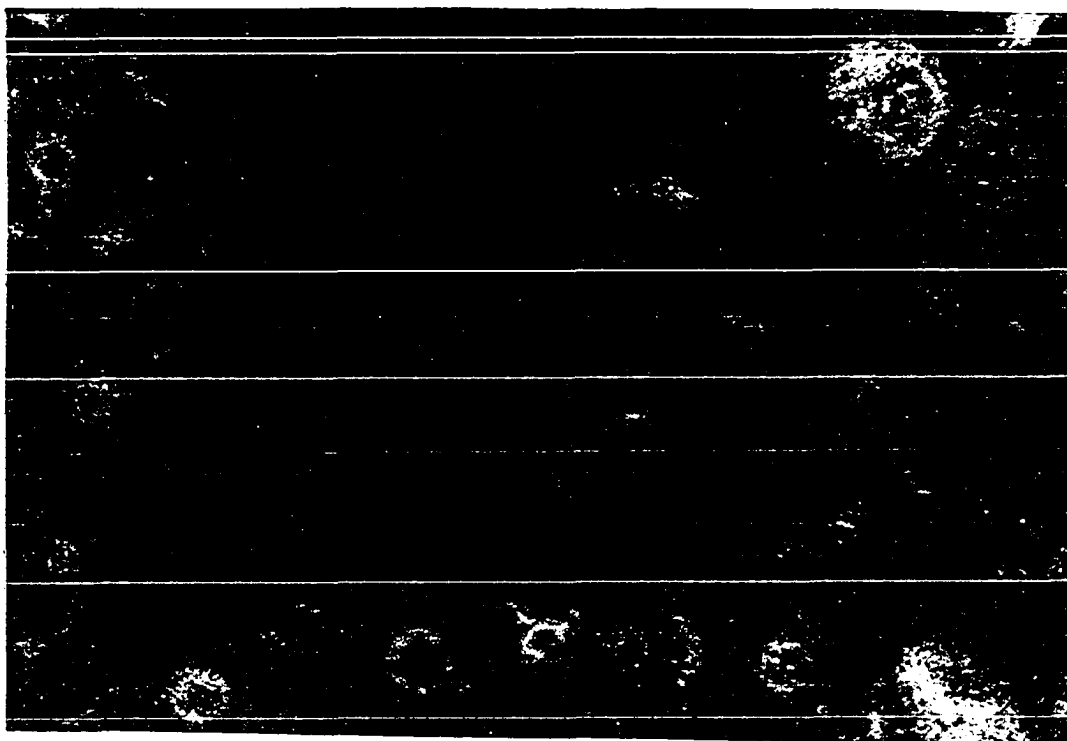
Figure 9. Light micrograph of a 1/5/5 caseinate to water to
fat slurry (The only obvious structural details
apparent are air bubbles (a) trapped in the viscous
solution (100 X))
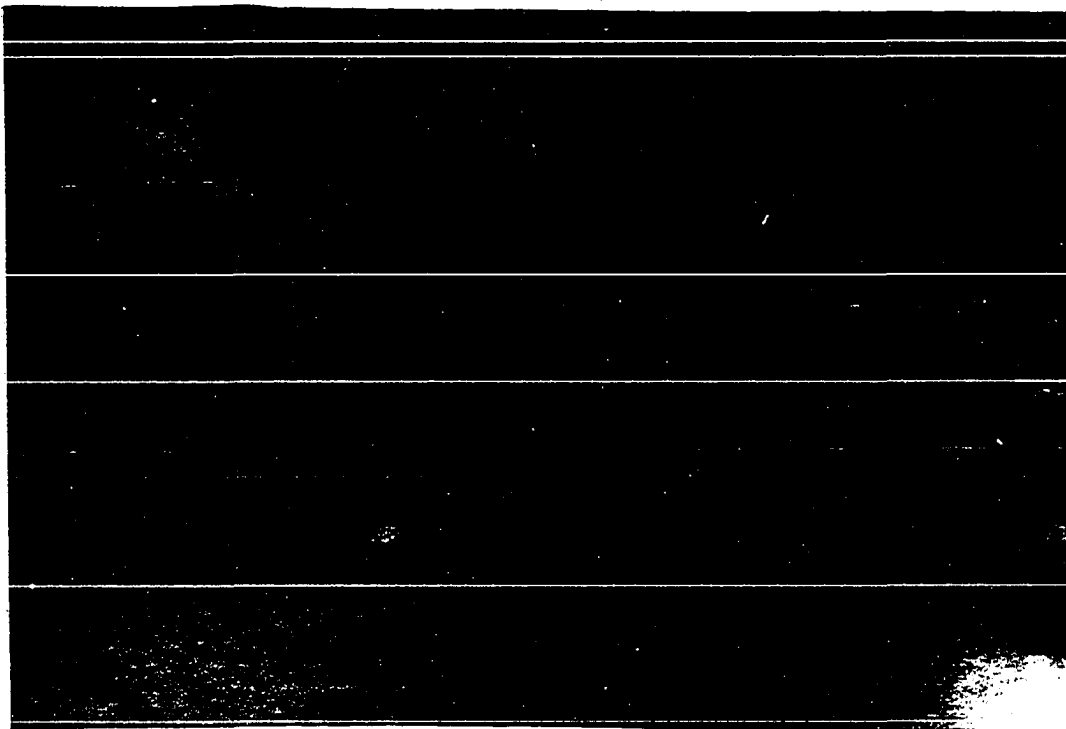
Figure 10.   Light micrograph of a 1/5/5 soy isolate to water
             to fat slurry (No distinct structures are
             visible (100 X))

Figure 11. Light micrograph of a 1/10/10 caseinate to water to fat slurry (100 X)

Figure 12. Light micrograph of a 1/10/10 soy isolate to water
            to fat slurry (100 X)

Figure 13. Light micrograph of a 1/25/25 caseinate to water
to fat slurry (Note the medium (m) and small (s)
cells which are becoming apparent (100 X))

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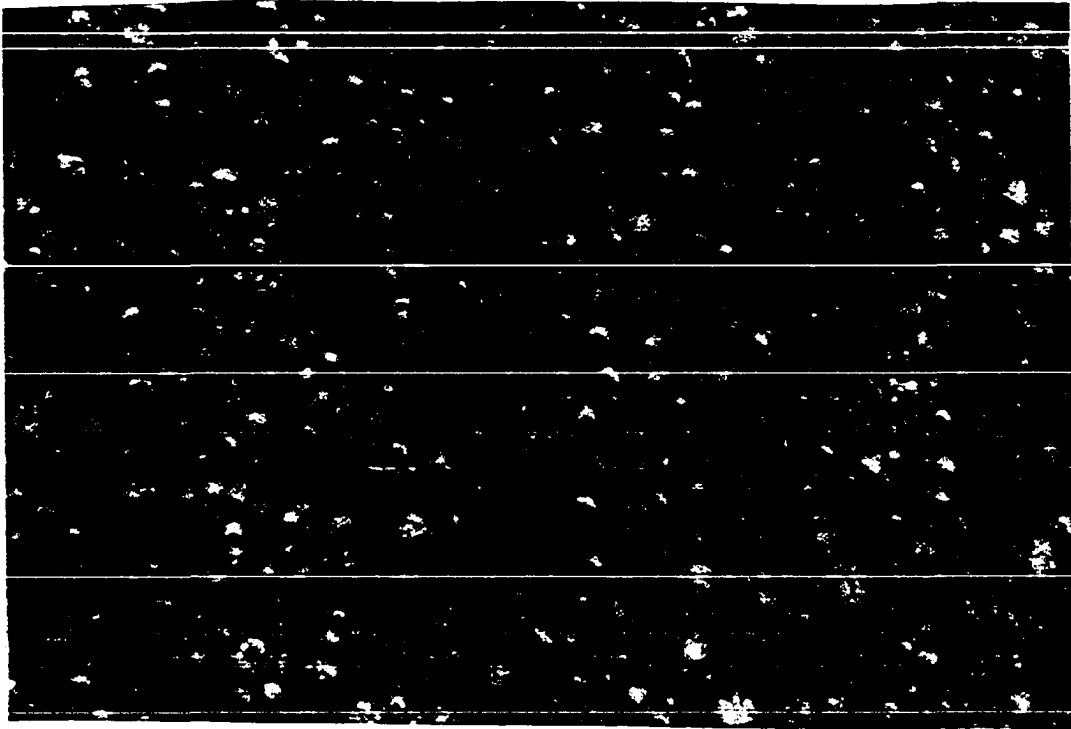
Figure 14. Light micrograph of a 1/50/50 soy isolate to
water to fat slurry (Although definite cell
structure is visible, a uniform size of cells is
apparent (100 X))

Figure 15. Light micrograph of a 1/50/50 caseinate to water to fat slurry (100 X)
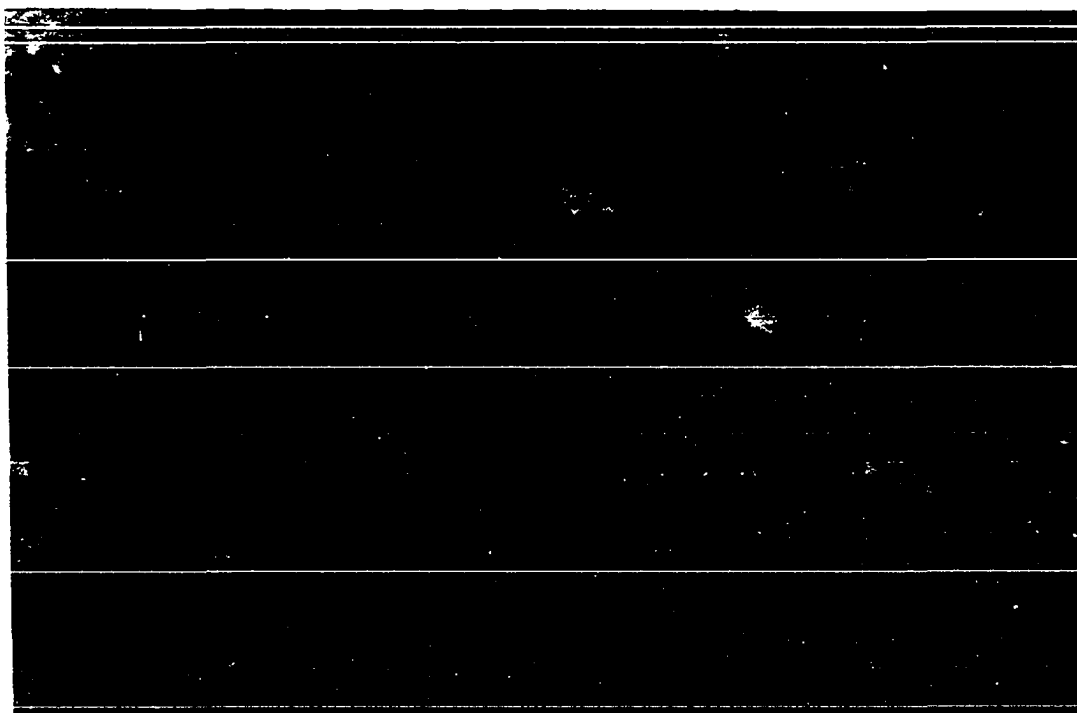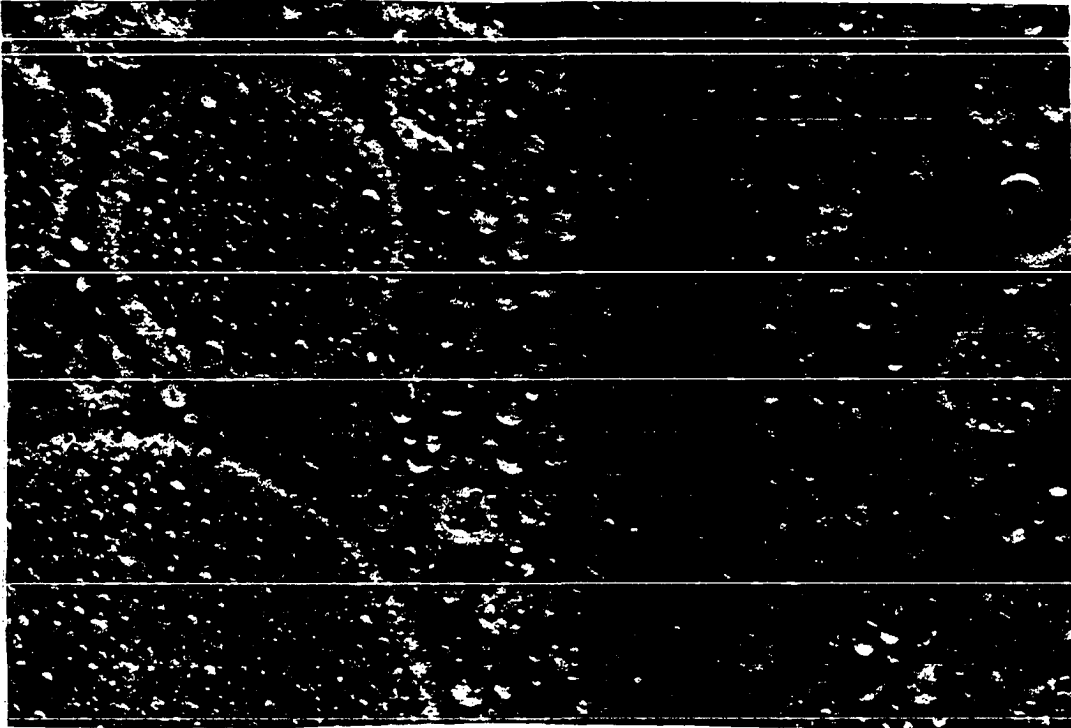
Figure 16.   Light micrograph of a 1/100/100 caseinate to water
to fat slurry   (Note the very large cell structure
visible in the lower left corner   (100 X))
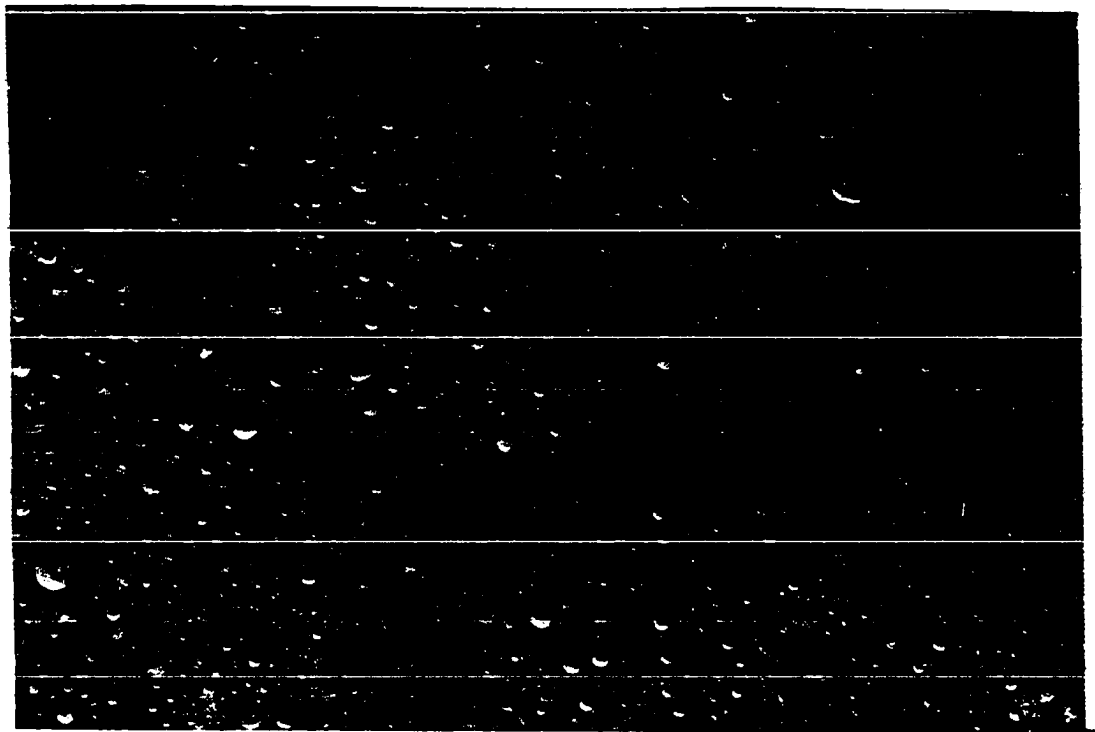
Figure 17. Light micrograph of a 1/150/150 caseinate to water to fat slurry (Extremely large cells (c) are formed. This slurry is becoming unstable (100 X))

point, the cells in which the lipid and water were dispersed
broke, releasing their contents (oil and water) to the
surrounding matrix.

At EC slurry ratios of 1/100/100 and 1/150/150 (Figure 18,
19) soy isolate slurries began to form cells, but did not
develop the larger cells seen at 1/150/150 levels in caseinate
slurries. At an EC slurry ratio of 1/200/200 (Figure 20) size
discrimination between the cells had begun to become obvious
and relatively large cells had formed.

At a ratio of 1/300/300, the soy stabilized EC slurry
provided a clear cut picture of the structure apparent just
prior to EC endpoint (Figure 21). In this picture, the
individual moisture filled cells are present complete with
Oil-Red-O stained lipid droplets. The center of the picture
consists of already collapsed cells with contents released to
the exterior. The point at which EC breakdown occurred was
characterized by breakage of the cell walls and release of
the contained oil droplets and water. In the case of both
isolated soy protein and caseinate EC measurements, EC
breakdown resulted in the formation of three distinct layers.
The layers were a lipid top layer, a protein layer and a
predominantly water lower layer.

Figure 22 is a schematic depicting the events which lead
to EC breakdown. In step 1, oil, protein and water combine to
form an indistinct mass (under low power magnification). The
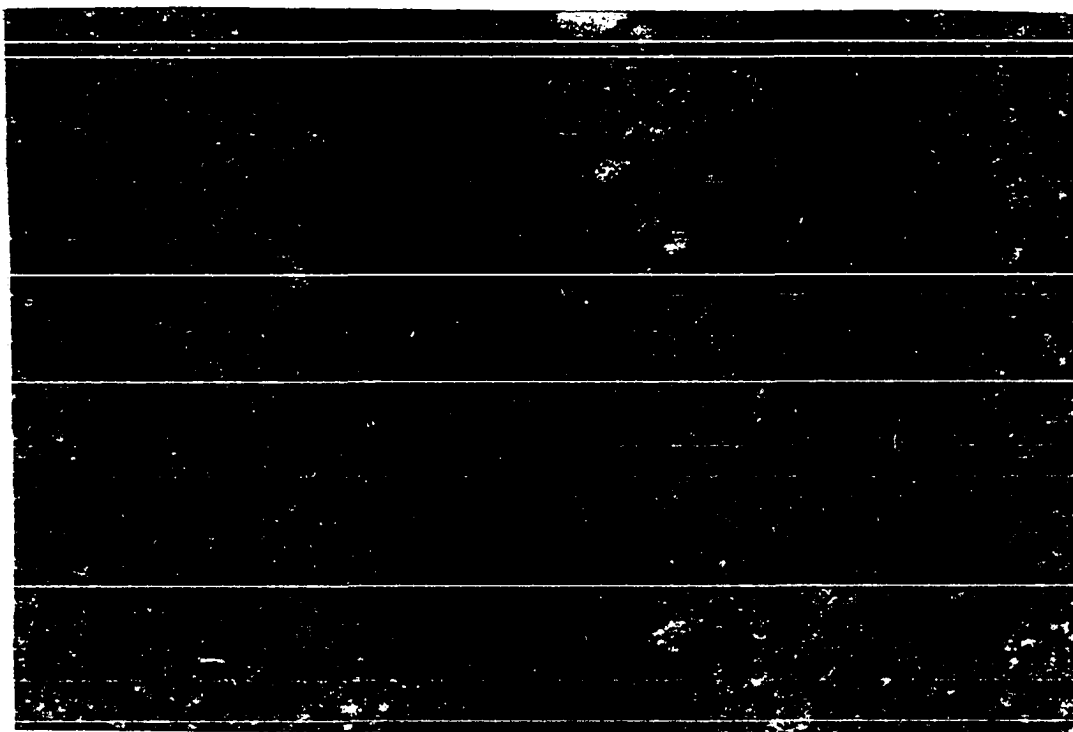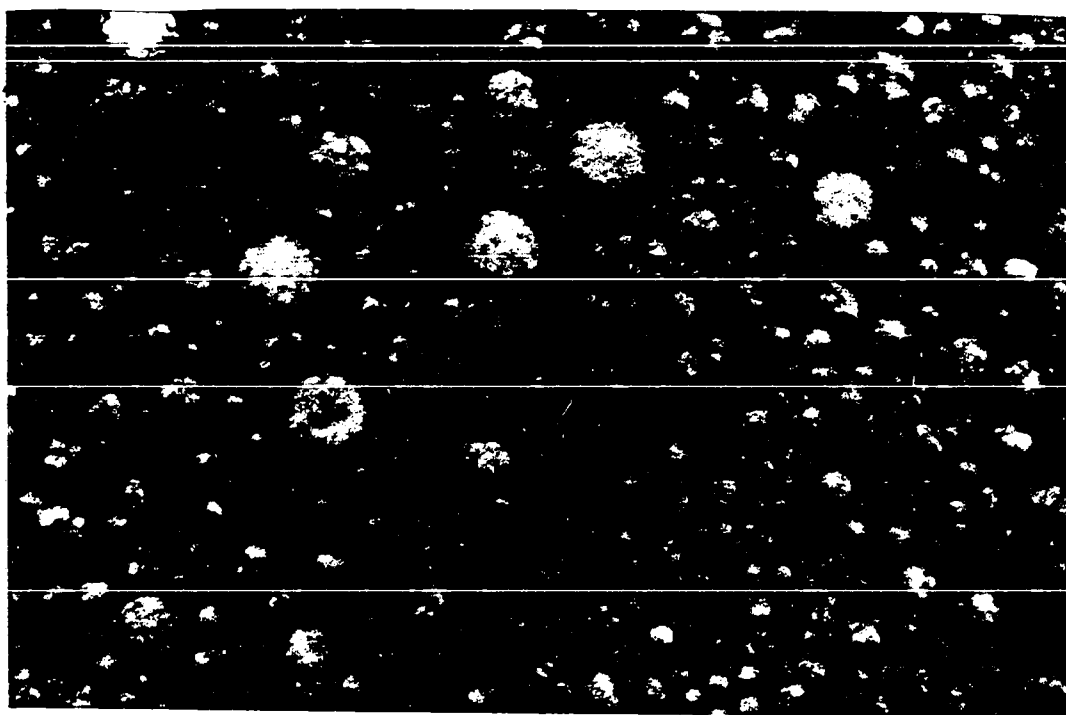
Figure 18. Light micrograph of a 1/100/100 soy isolate to
water to fat slurry (100 X)
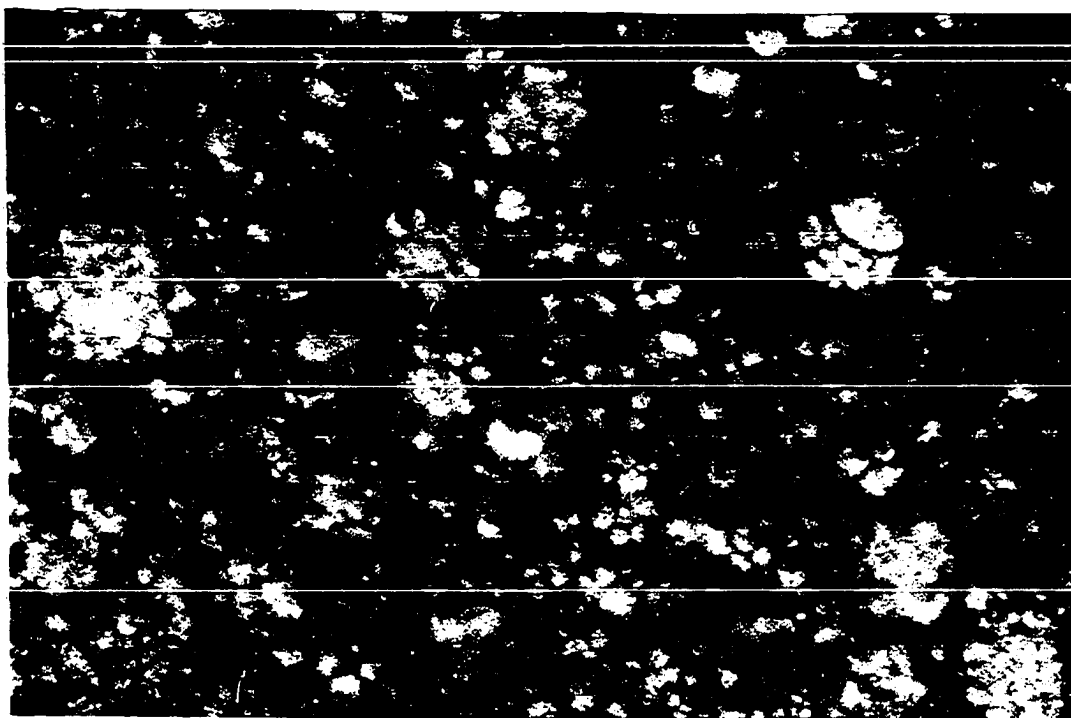
Figure 19.   Light micrograph of a 1/150/150 soy isolate to
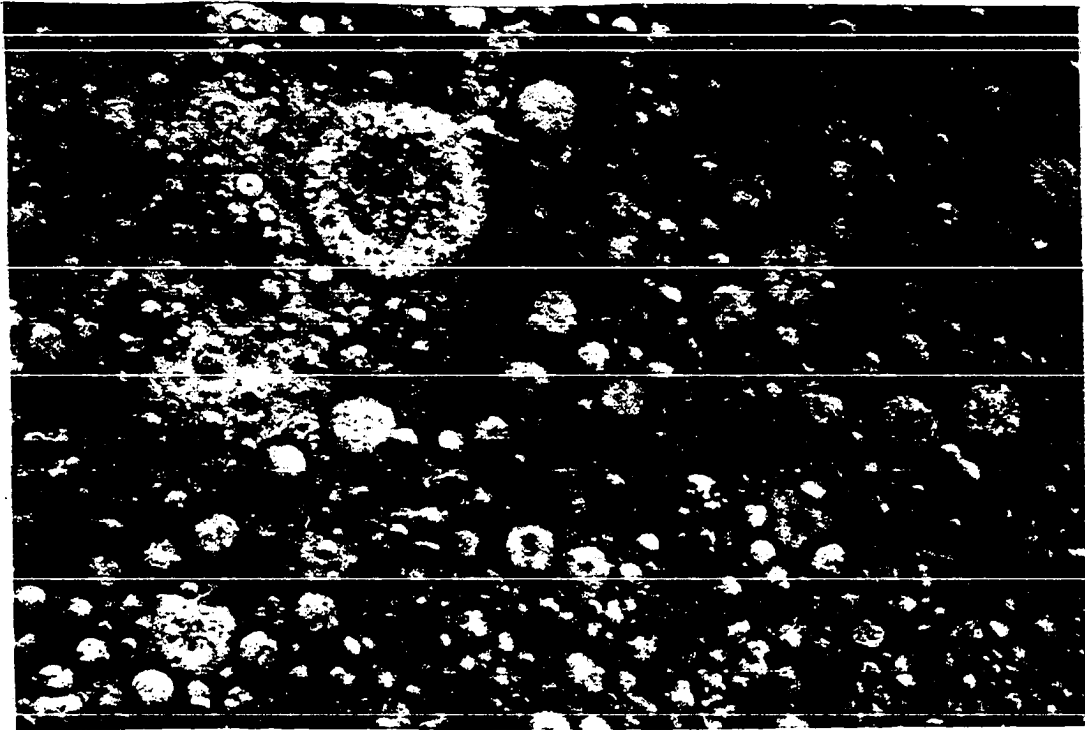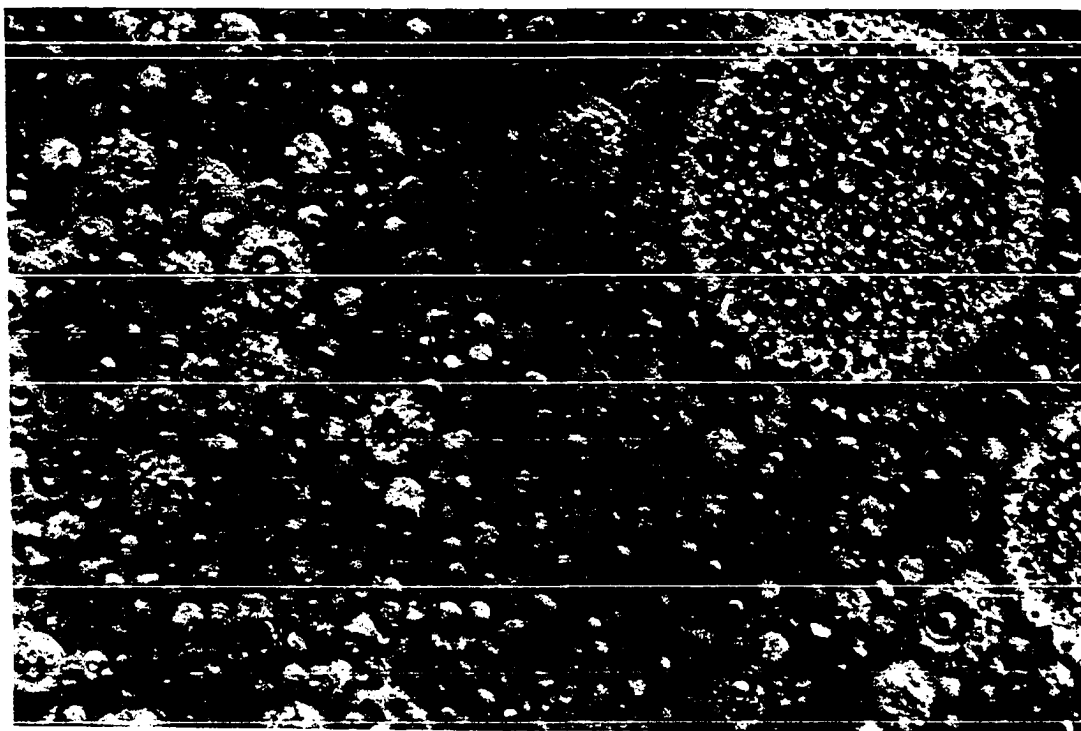   water to fat slurry   (100 X)
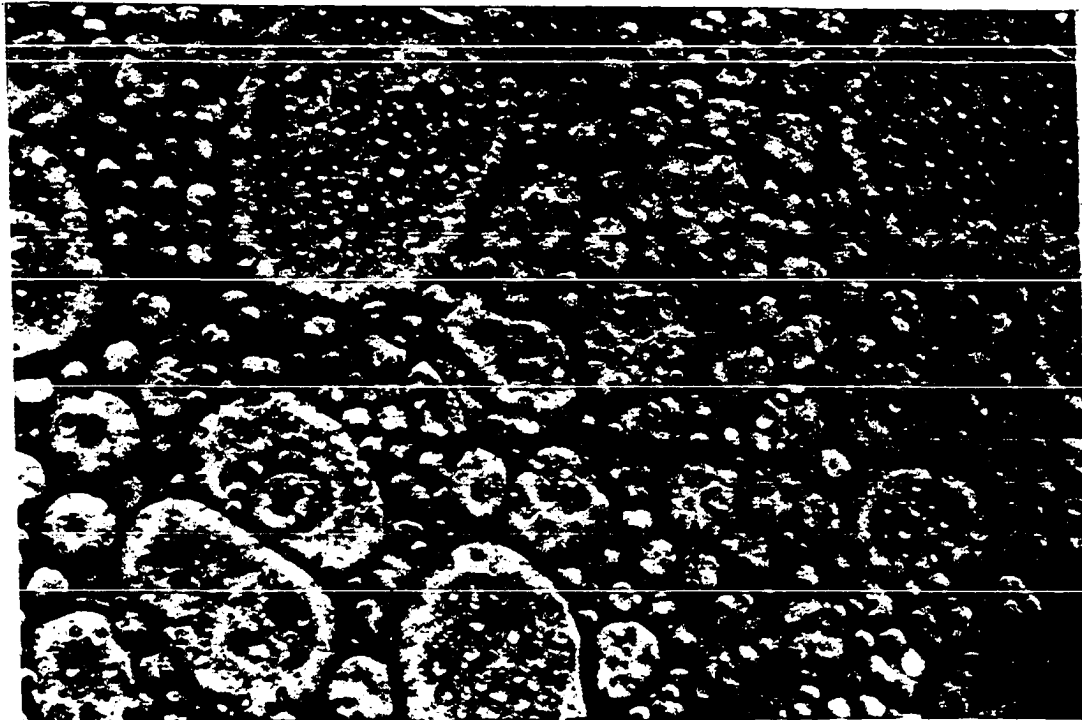
Figure 20. Light micrograph of a 1/200/200 soy isolate to
water to fat slurry (Note the formation of
definite cell structure (100 X))
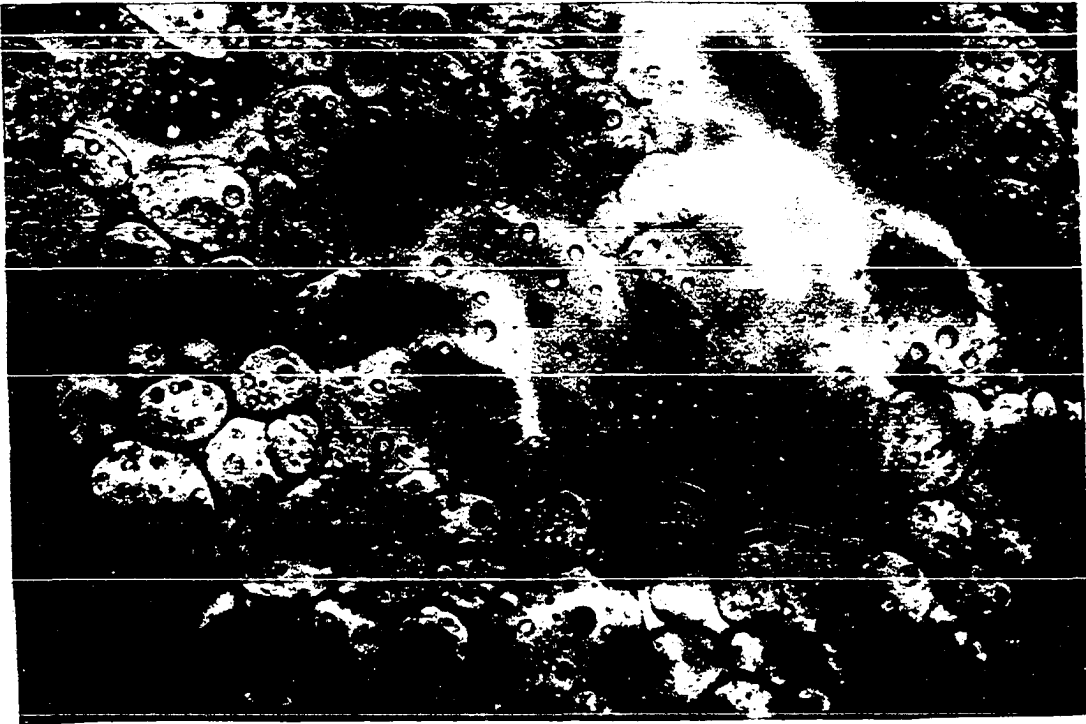
Figure 21. Light micrograph of a 1/300/300 soy isolate to
water to fat slurry (Note the presence of distinct
fat droplets present in water filled cells (a) and
released to the exterior (b) (100 X))

Step 1

Step 2

Protein
Water ⟶
Oil

Oil

Indistinct Mass

Formation of
Distinct Droplets

Oil

150

Step 5

Step 4

Step 3

Oil

Oil

Large unstable
cells, oil droplets
clearly visible

Large destabilized
cells, some internal
fat droplets are
visible
Oil

Formation of
multiple sizes of
droplets
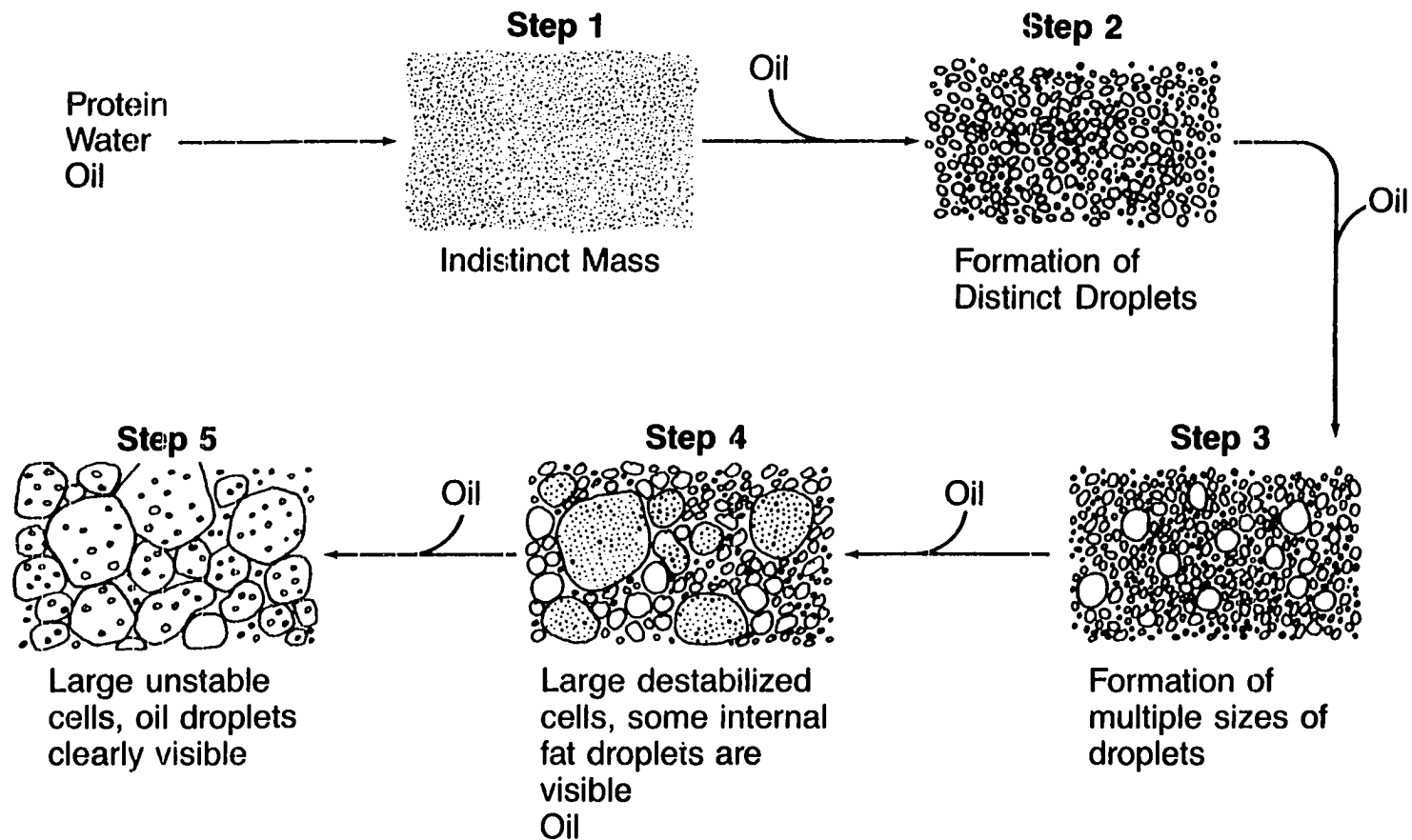
Figure 22. Structural events leading to emulsion capacity endpoint

structural development within this mass was undetermined.
Enough protein may be present to coat each fat globule forming
a classical emulsion (Becher, 1965). When additional oil was
added (step 2), distinct droplet or cell structure began to
develop. With increasing oil addition, cell sizes begin to
differentiate, forming assorted small, medium and large cells
(step 3). This step represented the beginning of
destabilization which occurred as large fat droplets were
formed. In step 4 after still more fat addition, very large,
unstable cells are present. Internal lipid droplets began to
be differentiated from the surrounding aqueous medium inside
the cell. As EC breakdown was approached in step 5, the lipid
droplets were easily identified dispersed within the aqueous
environment. At EC breakdown, the cell contents (water and
lipid) were released as cell protein walls were broken. By
staining portions of each of the three distinct layers formed
at the EC endpoint using Gill's hematoxylin, the middle layer
was found to contain the only appreciable amounts of protein.

The classical explanation of EC breakdown is that
insufficient protein is present to coat all the fat added.
The fat is then released to the external environment. This
release decreases the solution conductivity, concentrates
Oil-Red-O in the exterior or results in some other EC endpoint
event.

The term "emulsion" capacity is critical because it implies that the protein is located at the individual water/lipid interfacial surfaces. In this experiment, it was not possible to determine whether or not individual lipid droplets were protein coated. Two facts were able to be determined, however. First, lipid droplets were contained in an aqueous environment inside a larger cell structure. Second, EC breakdown was not determined by individual lipid droplets being released, but rather by the release of lipid droplets and water contained within enlarged cells. One point should be perfectly clear. If the description of the events resulting in EC breakdown is accepted, protein association at the lipid/water interface becomes of secondary importance in the context of emulsion capacity measurement. At best, this study indicates that "emulsion capacity" is a misnomer; at worst, it means emulsion capacity is worthless as an indication of protein to lipid interaction.

To determine what EC measurement does measure, the factors presented earlier must be re-examined. Studies of assorted protein types indicated that soy isolate possessed an EC equal to if not greater than that of caseinate. It was obvious during fat mixture production that the fat binding character- istics of the two proteins were not equivalent. It was also indicated that the soy stabilized fat mixture was stabilized by protein to protein gel interactions whereas the caseinate mixture was stabilized by protein to protein and protein to

lipid interactions. If it is true that the protein to lipid interaction is not important at the EC endpoint, the ability of the protein to form protein to protein interactions would ultimately determine the EC of the product.

NaCl was not used in the soy stabilized fat mixtures studied. NaCl was found to be, however, very detrimental to EC measurement. NaCl reportedly inhibits both the initial soy protein unfolding or denaturation and protein to protein interaction or renaturation required for optimum functionality (Hermansson, 1979). If this report was accurate and NaCl indeed inhibits protein to protein interaction, a drop in soy EC (as was seen) would have to occur when NaCl was added. Likewise, since NaCl increases caseinate protein to protein interaction, caseinate functionality would be enhanced by NaCl.

Temperature affected the EC of both proteins by decreasing the EC as temperature increased. The decreases in EC are contradictory to the lack of change seen in the caseinate fat mixture and the improved stability of heated soy mixtures with increased temperature. In the soy fat mixtures, the heat present appeared to be sufficiently high to induce gelation of the soy protein. If the concept of individual protein film coated cells containing both water and lipid is accepted, a hypothesis concerning the action of heat upon the system can be proposed. It was calculated that in a closed system, if water

at 4 PSI (atmospheric pressure) and 31° C was heated to 43° C, an increase of 1400 PSI would occur (Morin, private communication, 1986). The EC test, however, is neither a static nor a closed system. It is a system in which individual water and lipid cells are constantly changing. Protein coated cells are formed, and the film layers combine and reform larger cells as more lipid is added. With increases in temperature, however, a more rapid enlargement is promoted because each specific amount of fluid exerts greater pressure on the protein film layers. As a result, the ability of the proteins in the film layer to interact is probably not decreased due to the addition of heat. Rather, the heat increases fluid pressure in the system which "uses up" the protein film or causes it to expand to its maximum at relatively lower quantities of added oil.

The medium (water or lipid) in which the protein was initially dispersed had essentially no effect on EC. As described earlier, in caseinate EC measurements at 1.0 M NaCl concentrations, a physical situation unlike EC breakdown occurred. Aside from this unusual phenomenon, however, no major difference was noted. Neither traditional EC explanations or the alternate EC mode of action explained in this discussion would indicate that either medium of dispersion should improve the protein's EC.

EC micrographs published by other authors appear to agree with the breakdown of protein coated water and lipid cells seen in this study (Smith et al., 1973; Tantikarnjathep, 1980; Regenstein and Regenstein, 1984). It is unknown why this mode of breakdown has not previously been suggested. In this study, EC was inconsistent in estimating the effectiveness of a protein for stabilizing lipid. At times protein emulsification effectiveness has been accurately measured using EC (Carpenter and Saffle, 1964; Webb et al., 1970), at other times not (Mattil, 1971; Puski, 1976). The reason for this inconsistency is because EC measurement appears to measure a functional property of protein, but not necessarily the ability to interact between a water and lipid surface. Rather EC appears to estimate the ability of a protein to remain self-associated with increased expansion. A more appropriate term defined by the EC measurement may be protein elasticity.

In this discussion, soy isolate and caseinate fat stabilizing ability could not be predicted using EC measurement. The lack of consistency has been a common complaint concerning model systems in general. Unfortunately, complex food system production includes the addition of several confounding factors which influence the effect of the protein. The use of individual proteins to produce fat mixtures may provide a solution to the problem. By producing fat mixtures, the functionality of a protein can be examined in a system in

which relatively few interfering ingredients are present. In addition, the protein to water to fat composition of the fat mixture more closely simulates an actual food system than does the composition of an EC slurry or other model system.

Sausage Production Incorporating Fat Mixtures

Two types or classes of sausage products were produced using either soy stabilized fat mixture, caseinate stabilized fat mixture or 50% fat pork trimmings as the fat source. The two classes of sausages, coarse ground cotto salami and emulsified bologna were chosen in order to compare the effect of fat mixtures on varying textural and sensory characteristics.

Fat mixture characteristics

Prior to the manufacture of the sausages, the fat mixtures were produced according to the optimal production scheme determined in the first portion of this research. Table 30 displays the average characteristics of the two fat mixtures used in this study. Significant differences were not seen in either moisture or fat content between the two fat mixtures; however, two of the fat mixture characteristics were significantly different. First, the soy stabilized fat mixture demonstrated much more resistance to flow when compared to caseinate stabilized fat mixtures. This was earlier assumed to be the result of increased protein structural (gel) formation in the soy stabilized fat mixture. Second, as seen previously, the caseinate stabilized fat mixtures exhibited a slightly

Table 30. Effect of protein type on fat mixture characteristics[a]

Protein	Moisture (%)	Fat (%)	Instron Average (kg)	Emulsion Stability[b] (ml)	pH
Sodium Caseinate	50.3[c]	40.1[c]	4.8[c]	0.78	6.66[c]
Isolated Soy Protein	49.7[c]	38.3[c]	13.8[d]	0.03	6.73[d]

[a] $n = 9$ values per mean.

[b] Emulsion stability was significantly different ($P < 0.06$).

[c,d] Means within each column having different superscripts are significantly different ($P < 0.05$).

lower pH than soy stabilized fat mixture. Soy stabilized fat mixture was not significantly more stable than the caseinate mixture at $P<0.05$; it was different at the $P<0.06$ level, however. In Rongey stability measures of the three soy stabilized fat mixtures produced, 0.1 ml of lipid of a possible 40 ml was released. The caseinate fat mixture was somewhat less stable than previously seen. Even so, a fat mixture with an average fluid release of less than 1.0 ml/30 gm is essentially stable.

Raw product characteristics

The proximate composition of the raw and cooked product is seen in Table 31. The raw fat content of these products did not display any differences which were statistically significant. Bologna products possessed greater moisture content than their cotto salami counterparts. This was attributed to the addition of 3% water in the bologna formula. Products made incorporating stabilized fat mixtures displayed higher moisture contents compared to the products made using 50% fat pork trim. This resulted from the use of fat mixtures which had a slightly higher moisture content than expected.

After smokehouse processing, the products reached the desired target of approximately 25% fat. It appeared as though somewhat lower moisture losses occurred during thermal processing in the cotto salami which was produced using soy fat mixture. This was evident from the increase of only 1% in

Table 31. Effect of fat mixture addition on the raw and cooked compositional characteristics of bologna and cotto salami[a]

Product[b]	Raw Moisture Content (%)	Raw Fat Content (%)	Cooked Moisture Content (%)	Cooked Fat Content (%)
Control Bologna	$59.4^{d,e}$	23.6	$56.7^{d,e}$	25.4^{c}
Caseinate Bologna	61.6^{c}	24.6	58.4^{c}	26.7^{b}
Soy Bologna	$60.8^{c,d}$	23.0	$57.8^{c,d}$	$25.1^{c,d}$
Control Cotto Salami	56.4^{f}	23.6	53.8^{f}	$25.4^{c,d}$
Caseinate Cotto Salami	59.6^{d}	23.0	55.3^{e}	$26.0^{b,c}$
Soy Cotto Salami	57.9^{e}	22.9	55.8^{e}	23.9^{d}

[a] $n = 9$ observations per mean.

[b] Control denotes the addition of no fat mixture. Caseinate or soy denotes the use of caseinate or soy isolate stabilized fat mixtures, respectively.

[c,d,e,f,g] Means within each column having different superscripts are significantly different ($P < 0.05$).

relative fat content of the soy cotto salami compared to 1.8 to
3.0% increases in the other products. In addition, the cooked
moisture content declined only 2.1% from the raw product in soy
cotto salami, compared to 2.8 to 3.7 seen in the remaining
products.

Comparing the yield immediately following cooking, (SKYD)
and the final product yield after cooking and cooling (SSYD)
(Table 32), the cotto salami produced using soy stabilized fat
mixture demonstrated significantly greater yields when compared
to the remaining products. Products utilizing caseinate
stabilized fat mixtures tended to possess lower cooking yields
compared to the other products. These results will be
discussed together with the emulsion stability results.

Sausage batter stability

The Rongey stability of the raw meat batters is shown in
Table 33. A very interesting feature concerning the raw
product stability was that a large portion of the total loss in
every product was moisture relative to fat or solids. In one
case, fat loss was also high. This case occurred in the
product in which the fat was not finely chopped. By examining
Figure 23, a thin section sample of the control cotto salami,
and Figure 24, a thin section sample of soy stabilized cotto
salami, an insight into the stability phenomenon can be gained.
The fat globules formed by grinding and mixing are very large.
In contrast, in soy stabilized cotto salami, the fat was evenly

Table 32. Effect of fat mixture addition on smokehouse
yield after smoking (SKYD) and 18 hours of
cooling (SSYD)[a]

Product[b]	SKYD (%)	SSYD (%)
Control Bologna	92.5[d]	90.8[d,e]
Caseinate Bologna	91.5[c]	89.5[c]
Soy Bologna	92.6[d]	91.0[e]
Control Cotto Salami	92.7[d]	91.4[e]
Caseinate Cotto Salami	92.4[d]	90.4[d]
Soy Cotto Salami	93.9[e]	92.4[f]

[a]n = 3 observations per mean.

[b]Control denotes the addition of no fat mixture.
Caseinate or soy denotes the use of caseinate or soy
stabilized fat mixtures, respectively.

[c,d,e,f]Means within each column having different
superscripts are significantly different (P < 0.05).

Table 33. Effect of fat mixture addition on the Rongey stability and extrusion values of raw product batters[a]

Product[b]	Emulsion Stability (ml/30 g)				Extrusion (kg)
	Moisture	Fat	Solids	Total	
Control Bologna	3.8^d	0.1^c	0.2^c	4.1^e	2.6^c
Caseinate Bologna	6.0^f	0.0^c	0.2^c	6.2^d	$4.0^{d,e}$
Soy Bologna	3.1^c	0.1^c	0.1^c	3.3^e	$4.0^{d,e}$
Control Cotto Salami	5.3^e	5.2^f	0.1^c	10.7^b	3.5^d
Caseinate Cotto Salami	6.9^g	0.9^d	0.2^c	8.0^c	3.4^d
Soy Cotto Salami	4.0^d	1.6^e	0.1^c	5.7^d	4.3^e

[a]n = 9 observations per mean.

[b]Control denotes the addition of no fat mixture. Caseinate or soy denotes the use of caseinate or soy isolate stabilized fat mixtures, respectively.

[c,d,e,f]Means within each column having different superscripts are significantly different ($P < 0.05$).
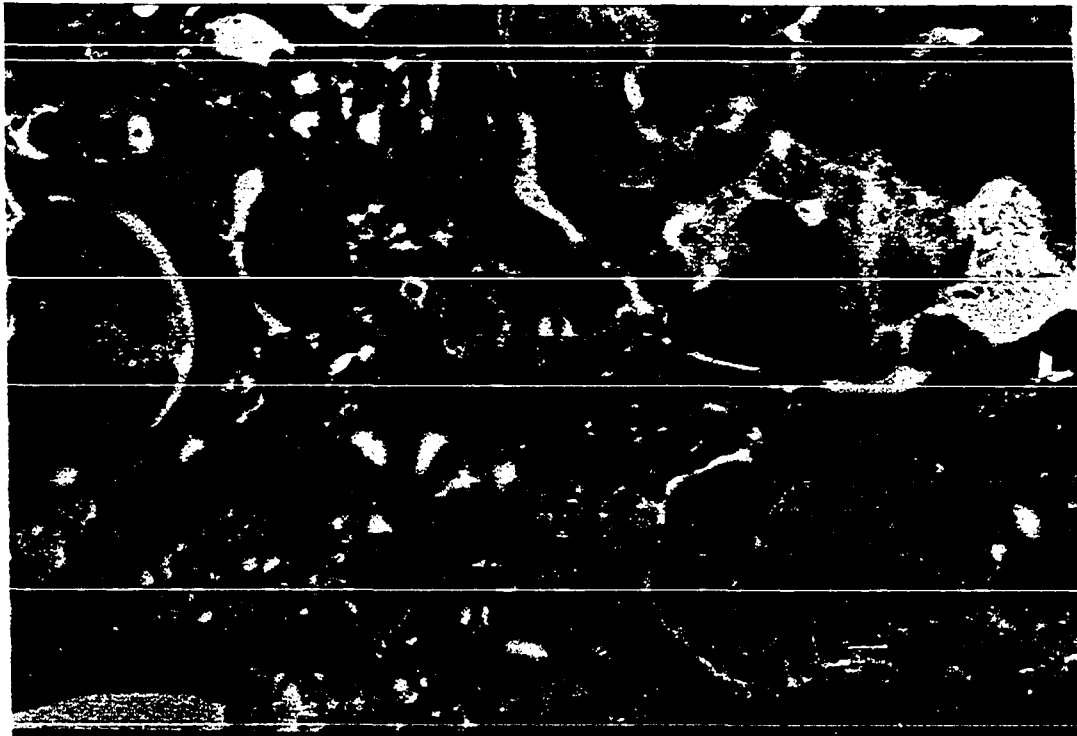
Figure 23. Light micrograph of the control cotto salami
(Note the large fat particle (a) located in the
upper right corner (150 X))
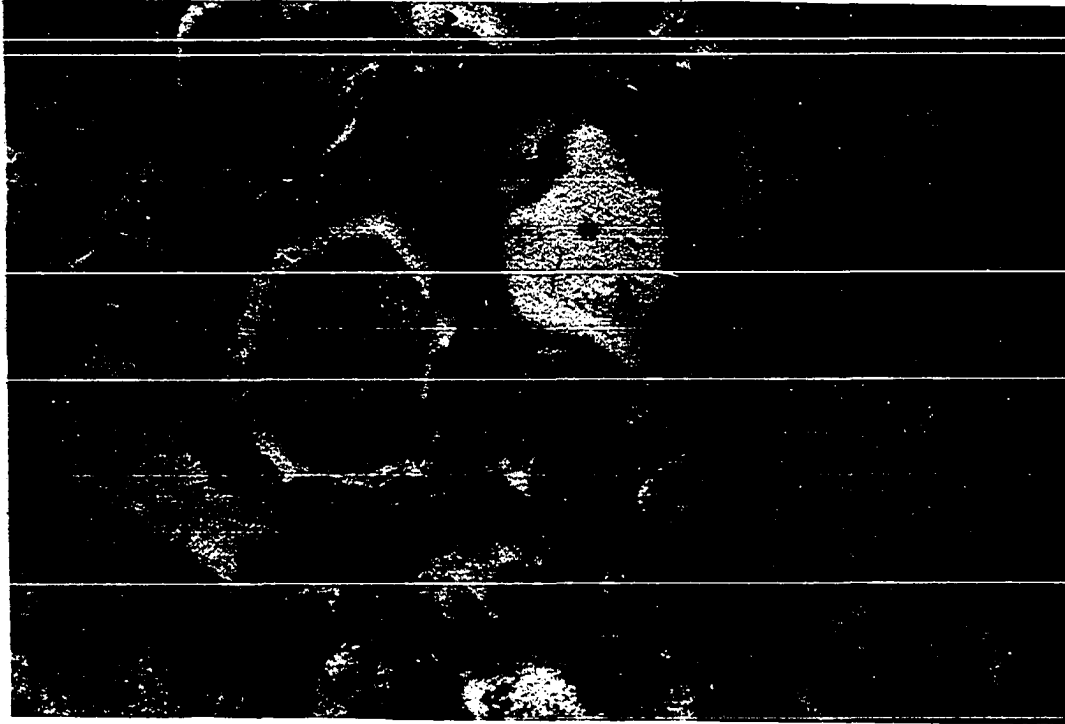
Figure 24. Light micrograph of cotto salami made using soy
            stabilized fat mixture as the fat source (Pockets
            of fat mixture (f) and water (w) are trapped
            within the protein matrix (p) (150 X))

distributed in a fine protein matrix. In all products using fat mixtures, the fat remained extremely stable during the Rongey stability heat treatment.

Another interesting feature of the Rongey evaluation was the amount of moisture lost from the product. In both the cotto salami and bologna, the products with caseinate stabilized fat mixture added had greater moisture losses than the regular or the soy fat mixture products. This observation indicated that the incorporation of the caseinate stabilized fat mixture diluted or destabilized the meat matrix resulting in an overall decrease in the water binding ability of the product. The lower yields in the product using caseinate stabilized fat mixtures (Table 32) probably resulted from increased moisture loss.

Table 34 presents the effect of product type and the fat source used on objective texture analysis. Two main features regarding peak hardness were apparent. First of all, bologna resulted, regardless of fat source, in greater peak hardness than its cotto salami counterpart. Secondly, the products which incorporated fat mixtures resulted in considerably lower hardness values during compression.

The measure of cohesiveness indicated that no difference in cohesiveness existed between the different fat sources when chopped into a bologna product. Cotto salami, on the other hand, demonstrated less cohesiveness in the two products which

Table 34. Effect of fat source and product type on
objective sensory estimates[a]

Product[b]	Hardness	Cohesiveness	Elasticity
Bologna	63.5[f]	44.7[f]	8.8[e]
Caseinate Bologna	33.2[d]	47.3[f]	8.6[e]
Soy Bologna	45.4[e]	45.0[f]	8.9[e]
Cotto Salami	54.0[e,f]	32.0[e]	8.1[d]
Caseinate Cotto Salami	20.3[c]	20.7[c]	7.4[c]
Soy Cotto Salami	29.1[c,d]	24.7[d]	8.1[d]

[a]n = 9 observations per mean.

[b]Control denotes the addition of no fat mixture.
Caseinate or soy denotes the use of caseinate or soy
isolate stabilized fat mixtures, respectively.

[c,d,e,f]Means within each column having different
superscripts are significantly different (P < 0.05).

included fat mixtures. Of the products with added fat
mixtures, caseinate stabilized cotto salami was less cohesive
than soy stabilized cotto salami. All cotto salami products
exhibited much lower cohesiveness than the bologna products.
The hardness and cohesiveness values indicated that the
chopping of the trim for a bologna type product together with
salt-soluble protein extraction results in a large increase in
internal structure formation.

A relatively small difference in product elasticity was
seen betwen any of the products. Cotto salami with caseinate
fat mixture displayed somewhat less elasticity than the
remaining cotto salami products. All the cotto salami samples
were lower in elasticity than the bologna samples. No
differences in elasticity between bologna samples were noted.

Objective color analysis

Two main effects were seen in the results of Hunter Lab
lightness evaluation (Table 35). In all cases, the bologna
product was determined to be relatively lighter than the cotto
salami product. In addition, product using a stabilized fat
mixture as the fat source was significantly lighter than the
products incorporating all meat trim. Neither fat mixture
resulted in a product significantly lighter than the products
made using the other fat mixture.

Hunter redness or A values were not significantly
different when the two products were compared (Table 35).

Table 35. Effect of fat mixture addition on the Hunter Lab color evaluation of bologna and cotto salami[a]

Product[b]	Hunter L	Hunter a	Hunter b
Control Bologna	58.5[e]	8.7[e]	8.8[d]
Caseinate Bologna	62.2[f]	6.4[c]	9.9[e]
Soy Bologna	61.3[e,f]	7.2[d]	9.7[e]
Control Cotto Salami	45.5[c]	8.7[e]	6.6[c]
Caseinate Cotto Salami	54.4[d]	7.4[d]	8.9[d]
Soy Cotto Salami	54.0[d]	7.2[d]	8.9[d]

[a]n = 9 observations per mean.

[b]Control denotes the addition of no fat mixture. Caseinate or soy denotes the use of caseinate or soy isolate stabilized fat mixtures, respectively.

[c,d,e]Means within each column having different superscripts are significantly different ($P < 0.05$).

Once again, however, fat mixtures in products resulted in significantly lower Hunter a values than products which incorporated only meat trim. Products incorporating fat mixtures were consistently less red than products made without fat mixtures.

Hunter B values followed similar trends as the Hunter L values. Both products produced using either fat mixture exhibited a greater relative yellowness than the two products made using all meat trim. In addition, bologna products were significantly more yellow than their cotto salami counterparts.

The results of the sensory evaluation of sausages made with or without fat mixtures are shown in Table 36. A seven point hedonic scale was used in each case. Both products made with all beef trim were rated high in flavor, texture and overall quality. The products incorporating soy stabilized fat mixture were rated poor in all features. The bologna products incorporating soy stabilized fat mixture were rated very low in flavor though somewhat better in texture. The poor flavor overshadowed the texture for a low overall rating. Cotto salami produced using soy stabilized fat mixture was not rated as low in flavor as bologna. The textural assessment of soy stabilized cotto salami was similar to soy stabilized bologna. Bologna produced using caseinate stabilized fat mixture received a higher rating than soy stabilized bologna. The sensory texture measurement results were interesting because

Table 36. Effect of fat mixture addition on the sensory evaluation of bologna and cotto salami[a]

Product[c]	Flavor	Texture	Overall
Control Bologna	4.8^g	4.7^g	4.7^e
Caseinate Bologna	4.2^f	4.1^f	4.0^d
Soy Bologna	2.7^d	3.2^e	2.8^c
Control Cotto Salami	5.1^g	5.0^g	5.0^e
Caseinate Cotto Salami	3.4^e	2.3^d	2.8^c
Soy Cotto Salami	3.2^e	3.1^e	3.1^c

[a] n = 34 observations per mean.

[b] Product rated using a 7 point hedonic scale (1 = worst, 7 = best).

[c] Control denotes the addition of no fat mixture. Caseinate or soy denotes the use of caseinate or soy stabilized fat mixtures, respectively.

[d,e,f,g] Means within each column having different superscripts are significantly different (P < 0.05).

objective measurement of the texture indicated that incorporation of the soy stabilized fat mixture produced a harder and more cohesive bologna than caseinate stabilized fat mixture. It is the author's opinion that the soy flavor apparent in the soy stabilized bologna instilled a greater dislike of the product which translated into poorer texture and overall ratings from the untrained panel. Caseinate stabilized cotto salami was rated poor for flavor and very poor for texture.

Fat Mixture Interaction with Meat Protein

The use of stabilized fat mixtures in a sausage product is a relatively new procedure. Addition of non-meat proteins in sausages has, as reviewed earlier, existed for some time. Addition of non-meat protein and fat in an already stabilized form, however, results in a situation not directly comparable to the addition of non-meat proteins alone. Of the three articles discussing fat mixtures, only Zayas (1985) incorporated stabilized fat mixtures using non-meat proteins into comminuted meat products.

When the decision was made to use protein stabilized fat mixtures in meat products for this study, it was arbitrarily decided to replace more than 30% of the fat with fat mixture. (DMV recommends adding only 30% of the fat as fat mixture.) There were several reasons for this. First, the process of making the stabilized fat mixture is, although simple, still

a process which requires a considerable amount of time and labor. The purpose of this process is defeated if the use of additional fat trim prevents the advantages described earlier such as total compositional control. Secondly, the goal was to develop a product exhibiting a maximum stability level. It was felt that by stabilizing as much fat in the product as possible, the most stable product would be produced. Thirdly, incorporation of a significant quantity of stabilized fat mixture into the product was expected to most effectively test the use of a fat mixture in a sausage product.

Products used in this study enabled the examination of several factors in sausage manufacture. Bologna permitted the study of fat mixtures in an "emulsified" meat product at a much higher level than seen in previous fat mixture studies (Zayas, 1985). The use of bologna also permitted the study of fat mixtures in a relatively spice free or bland product. (One primary concern about the use of soy protein in meat products is the presence of "beany" or green flavors.) It was important to ascertain the influence of the soy flavor on the usefulness of soy stabilized fat mixtures. Cotto salami provided a product considerably different than bologna in which to test the use of fat mixtures. Cotto salami is a ground product with a coarser internal structure and contains a higher level of spices than bologna.

These two products did not by any means represent every type of meat product. Most noticeably missing were the fermented, dried and semi-dried products. The products used, however, did provide an estimation of the effect of fat mixture use in two widely different products.

Product stability

Since the primary purpose of using stabilized fat mixture is to improve product stability, this is the logical place to begin the discussion. Zayas (1985) indicated a 6-7% increase in product yield in product with stabilized fat mixture added compared to control product. Although not apparent in smokehouse yield, the Rongey stability measure of the raw sausage batters did indicate improved fat stability in products, especially cotto salami which incorporated fat mixtures. This increased fat stability is due to the characteristics of the protein which stabilize the fat. With meat products, it has been felt recently that although initially meat emulsions may stabilize fat prior to cooking, meat protein gelation maintains fat stability. Saffle (1968) commented on this:

> "In uncooked emulsion, the fat micelles were surrounded with protein...two important changes were noted for the thermally processed emulsion: First, the membrane surrounding the fat micelles has been disrupted, resulting in a number of definite pores or openings.

Secondly, the continuous phase of the emulsion has

been severely altered by thermal processing. The

matrix appeared highly disrupted, with the protein

being coagulated into dense irregular zones.... This

leads to pure speculation that when the membrane was

broken the matrix was too viscous or had 'set up' to

the point that the fat was 'trapped' and could not

come to the surface."

Carroll and Lee (1981) and Lee et al. (1981) also comment on
fat being found in finished product in irregularly shaped
pockets with stability determined largely by the protein
matrix. As stated previously in the review, meat gelation
begins at 33-36°C (Schweid and Toledo, 1981). At this
temperature, the fat in the emulsion is still expanding. This
results in the rupture of the emulsion film during thermal
processing (Smith et al., 1973). The caseinate and soy
proteins used in this study do not react to thermal processing
in the same manner as meat proteins. As a result, whether fat
is trapped in a soy isolate gel or caseinate emulsion, fat
expansion during thermal processing is not restricted to a
specific rigid size as is seen with meat proteins. If the
cells containing the fat remain flexible and do not rupture,
the fat should remain trapped, and as such, stabilized in the
matrix.

The light micrographs of the bologna produced using no fat mixture and caseinate stabilized fat mixture are compared in Figures 25 and 26, respectively. It is seen that the control product exhibits a more coarse protein texture with interspersed fat particles, while the caseinate stabilized product exhibits a much finer protein network with few definite fat pockets.

The situation with regular cotto salami was somewhat different. No emulsion was formed in the ground product. In cotto salami made using 50% fat pork trim, large fat globules were apparent as seen in Figure 23. These globules were weakly stabilized in the coarse matrix and appeared to express fat out of the product during thermal processing. The cotto salami incorporating soy stabilized fat mixtures (Figure 24) or caseinate stabilized fat mixtures (Figure 27) appeared to have distinct pockets of fat mixture which varied in size, but were much smaller than the control product's fat particles. It must be remembered that each of the fat mixture pockets seen in products made with stabilized fat mixtures was independently stable.

If protein to lipid interaction less important than protein to protein interaction for product stability, an explanation concerning the stability can be developed. In the products other than cotto salami made with 50% fat pork trimmings, a fine network of protein was seen. This protein
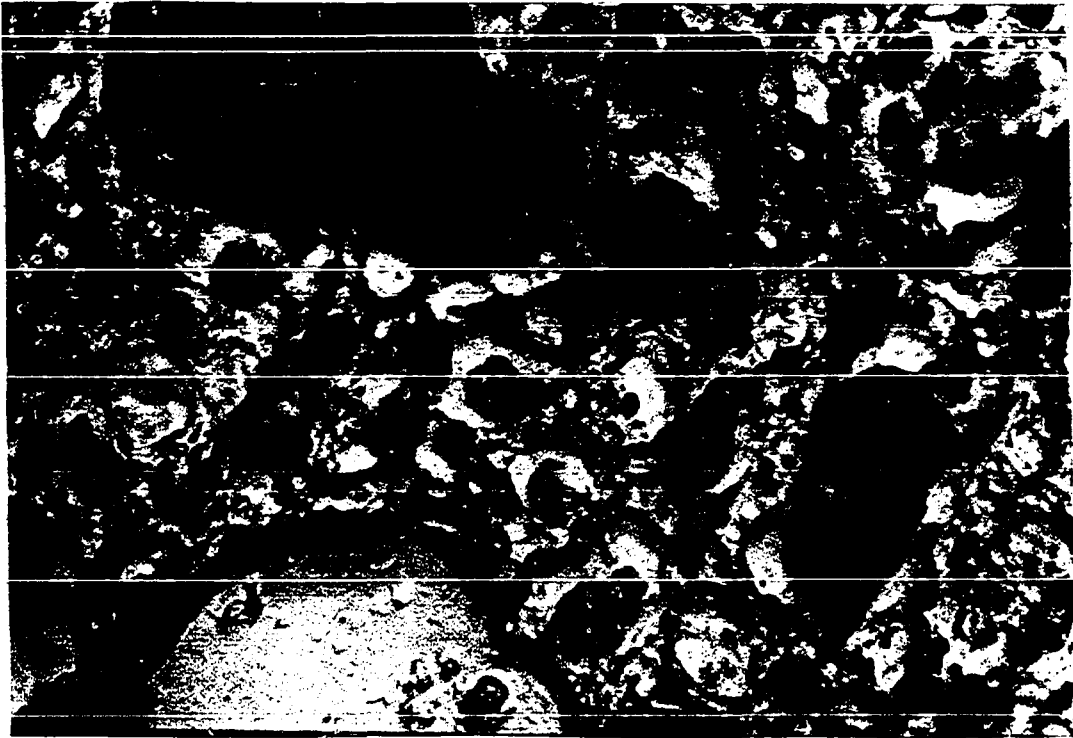
Figure 25. Light micrograph of bologna produced using 50% fat
pork trimmings as the fat source (Large and small
fat globules (f) are distributed in a protein
matrix (150 X))
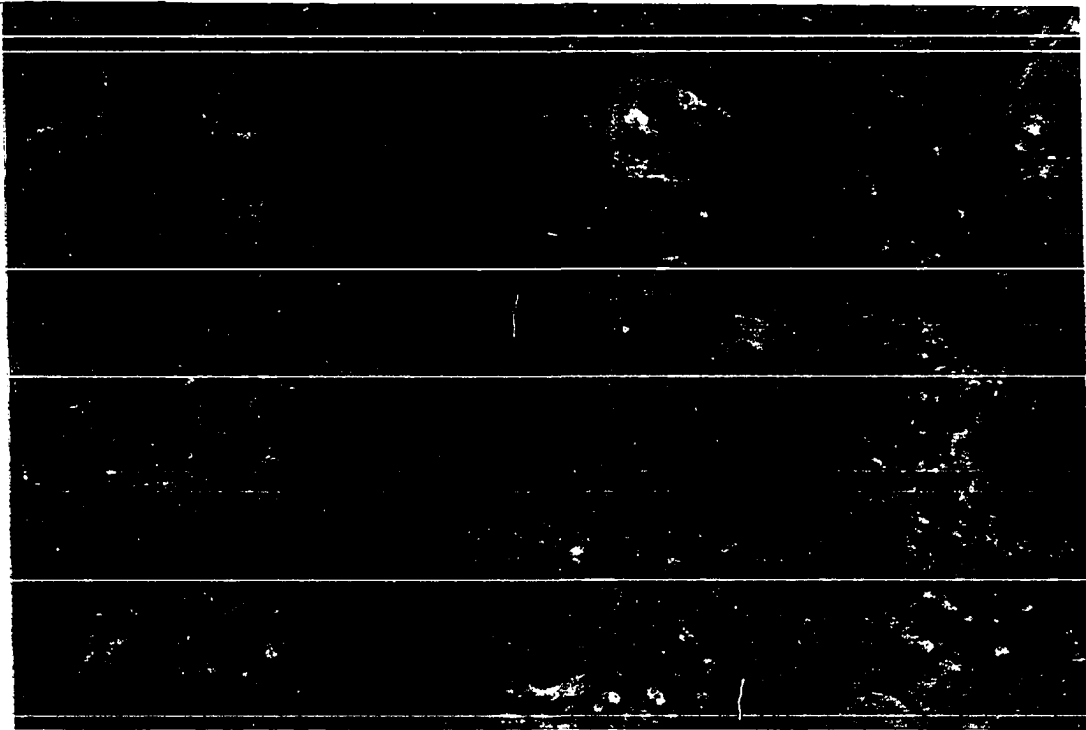
Figure 26. Light micrograph of bologna produced using
caseinate stabilized fat mixture as the fat
source (Distinct fat particles are difficult to
locate (150 X))
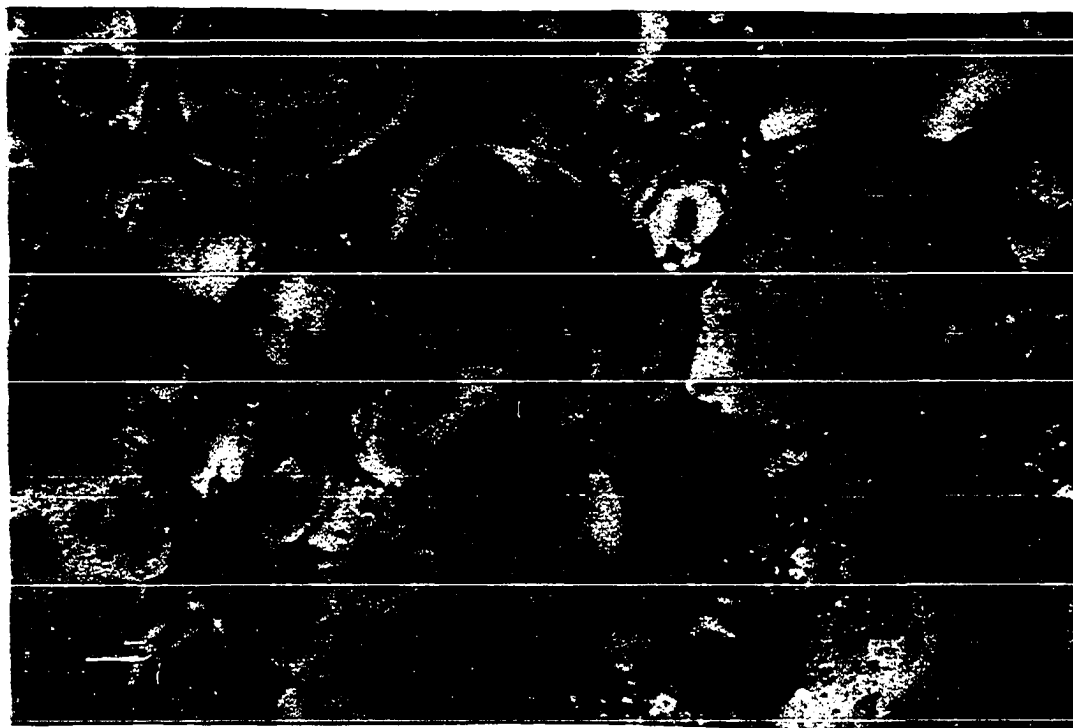
Figure 27. Light micrograph of cotto salami produced using
caseinate stabilized fat mixture as the fat
source (Fat mixture (f) is entrapped within the
meat mixture (150 X))

formed a very effective inhibitor to fat migration during
cooking. The finer, more complex the matrix that was formed,
the more difficult it was for fat migration to occur. Fat
would tend to become distributed throughout the matrix rather
than in discrete particles. This distribution appeared to
occur in fat mixtures. In products which have fat mixtures
included, it was evident that the fat particles, especially in
cotto salami, consisted of small masses of intact fat mixture
rather than fat globules.

Unfortunately, the sausages with fat mixture added did not
display a major improvement in product yields compared to the
control sausages. It is quite possible that the heat
processing levels used in this study were too low to initiate
excessive losses from the regular product. It is also possible
that the incorporation of so much fat mixture decreased the
effective stabilizing qualities of the meat protein.
Regardless, in this type of product processed in the method
described in this paper, no benefit in final product yield was
realized as a result of stabilized fat mixture addition.

Product texture

Product made using fat mixtures displayed a below average
level of textural acceptability. Fat mixtures were more
acceptable when added to the emulsion product than when
incorporated in the ground product. Once again Zayas (1985)
provides the only previous study of the textural properties of

meat products with fat mixtures added. Zayas concluded that cooked sausage products which contained fat mixtures stabilized by caseinate exhibited lower compression and elasticity than control products.

In most cases where non-meat protein has been added to a meat system, the resultant texture exhibited lower hardness, elasticity or cohesiveness values than all-meat products. Caldironi and Ockerman (1982) found sensory textural ratings lowered in 8 of 9 treatments in which combinations of plasma and globin proteins were added to bologna. Likewise Terrell et al. (1982) found textural decreases with blood proteins in frankfurters. Using sodium caseinate, Comer and Dempster (1981) found decreases in Ottawa texture measurement of 20 to 40% in wieners. Smith et al. (1973), Lauck (1975) and Sofos and Allen (1977) described emulsion products in which soy isolate addition decreased the textural parameters. Smith et al. (1973) did see an increase in textural preference when soy isolate with a high nitrogen solubility was used.

Both raw sausage batters containing soy stabilized fat mixture required a greater amount of force to extrude than sausages made using caseinate fat mixture or control product. In cooked product, however, both products, with stabilized fat added, required less force to compress than the control product. In general, products containing soy stabilized fat mixtures required more force to compress than products containing caseinate stabilized fat.

Two mechanisms can be suggested to explain the effect of fat mixture addition on product texture. The first mechanism deals with the alteration of meat protein matrix through the integral incorporation of non-meat proteins within the meat matrix. This mechanism, in essence, dilutes the meat protein matrix. Since the soy or caseinate proteins are not subject to the formation of a rigid structure during heat processing, as meat proteins are, the sausage structure will tend to be softer. As a result, the product would exhibit a decline in textural characteristics as fat mixture is added at greater levels.

A second mechanism of effects of fat mixture incorporation is that the fat mixture is distributed relatively intact in the final sausage product. Chopping or grinding finished fat mixtures was expected to decrease the stability of the stabilized fat mixture. This did not occur. As a result, there is no reason to assume that the individual pockets of fat mixture are broken down to any great extent. Therefore, discrete pockets of fat mixture may become embedded in the protein structure of the sausage. If this was the case, the texture of the final product should not be greatly different than the control meat product.

Neither mechanism mentioned is adequate to describe both the fat stabilization and the effects on textural characteristics of the sausage products. The most likely structure of the sausages containing fat mixtures is a

combination of the previous two mechanisms. The fat mixture remains intact while the protein stabilizing the fat mixture interacts with the meat protein present. The meat protein matrix is, as a result, diluted and also connected to a certain extent with non-meat proteins. Thee non-meat proteins are not as susceptible to heat treatment as are meat proteins, and during heating, the meat proteins coagulate, but the non-meat proteins do not, resulting in a sausage in which stiffening meat proteins interact with non-stiffening non-meat protein matrix. This mechanism not only results in a lessening of the overall texture development, but also may explain the decreased water stability when caseinate stabilized fat mixture was used.

Product appearance

Use of fat mixture resulted in a lighter, less red product color. Both fat mixtures possess a very white or light color. Substitution of fat mixture for 50% fat pork trim means that approximately 1/3 of the lean tissue has been removed from the product. Since 50% fat pork trim rather than beef trim was replaced, somewhat less than 1/3 of the total myoglobin content was removed. This decrease in myoglobin content explains the less dark, less red color seen in products incorporating stabilized fat mixture as the fat source.

When fat mixtures were added to products, no particle definition of the fat was apparent. This was because the fat mixture did not retain a given particle definition in the

sausage; rather, the fat mixture blended into the meat matrix. This was not a problem in bologna because none of the emulsion products exhibited particle identity. The cotto salami product, however, should have displayed clear fat particles distributed throughout the lean. Instead, lean particles could be seen in a lighter background. This was very unappealing from a sensory standpoint and presented a major drawback to the use of fat mixture in sausage products.

CONCLUSIONS

Several conclusions can be drawn from the research presented in this paper. The most important finding is that isolated soy protein could be combined with fat and water to produce a stable fat mixture. This mixture was at least as stable as caseinate stabilized fat mixture. The conditions used to produce the stable soy stabilized fat mixture are as follows:

1. Four and one-half parts of 90°C water (less 100 gm) and one part of Purina 500E (or 620T) isolated soy protein were chopped together for 60 sec under continuous steam heating.

2. Four parts of fat chunks were added to the soy-water mixture and chopped under steam for a total of 10 min. (A minimum chopping temperature of 48.9°C was required.)

3. At 8.5 minutes into the chopping cycle, the 100 gm of the remaining water, in which sodium nitrite was dissolved, was added.

It is important to note that opposed to the conditions for producing caseinate stabilized fat mixtures, very specific water content and minimum chopping temperatures were required to produce the soy stabilized fat mixtures. Soy isolate was able to stabilize only 50% of the fat (per unit weight of protein) that caseinate stabilized.

When compared to measuring protein functionality using fat mixtures, the model system emulsion capacity measurement was not particularly effective in measuring protein functionality. Temperature, protein type and addition medium all produced a different effect on apparent protein functionality in the emulsion capacity measurement than in fat mixture production. Further investigation suggested that emulsion capacity measurement is probably not directly related to the protein's ability to emulsify oil. Rather, emulsifying capacity measures a functional property involved with protein to protein interaction. Where this interaction is important to fat stabilization, emulsion capacity may mimic fat mixture results.

Fat mixtures closely approximate the simple formulations seen in model system analysis, but also closely approximate fat and water levels commonly found in food products. This feature suggests that fat mixture production may provide a good method for the estimation of protein functionality in food systems.

It was very evident that in 25% fat products a fat mixture could not effectively be substituted entirely for 50% fat pork trim. The primary problems associated with excessive fat mixture usage were related to texture. In bologna products, an increase in softness resulted from the use of fat mixtures. In cotto salami, the decrease in texture resulting from addition of fat mixture was very detrimental to product texture. Any

products with fat mixtures added were objectively lighter, less red and more yellow than control products. No improvements in smokehouse yield were seen in products with fat mixtures added. Rongey stability measurement of raw meat batters, however, indicated a potential fat stability improvement in products containing stabilized fat mixtures, but this was not apparent during smokehouse cooking of manufactured product.

SUGGESTIONS FOR FUTURE RESEARCH

Several aspects of this research deserve further examination. Most importantly, attempts to produce fat mixtures using other protein sources such as blood proteins, novel plant proteins and single cell proteins should be undertaken. This would determine if the process of fat mixture production is a viable method of estimating protein functionality in a simulated food system. Fat mixture production using other proteins may also provide an indication of the mechanism of fat stabilization in food products.

It would be extremely valuable to develop a method which distinguishes between protein to protein interactions and what appears to be protein to lipid interactions. The author feels, however, that protein to lipid interactions in food systems may actually be protein to protein interactions.

Results of this research suggest that two specific features of model systems require further study. First, the emulsion capacity test should be studied to determine what the test is actually measuring. The emulsion capacity test may well provide a valuable estimate of protein functionality unrelated to emulsification. What functional property is being measured and how the estimate can be used still remain to be determined. Second, other proteins should be tested to determine if the endpoint of EC is universally due to the disruption of large cells of fat and water as seen in this

experiment.

Further reserach is necessary to optimize the use of stabilized fat mixtures in sausage products. It was apparent that the fat mixtures produced in this research have their limitations for use in sausage products. In both products, the flavor and textural changes caused by fat mixture addition were undesirable. In bologna, simply reducing the amount of fat mixture added may result in acceptable texture. In bland products, it may be necessary to develop soy stabilized fat mixtures using soy isolates with even less "soy" flavor than Purina 500E (or 620T).

For use in ground products, a method is needed to produce a stabilized fat mixture which exhibits an independent particle structure. One possible method to accomplish this would be to hard freeze then mechanically disrupt the fat mixture. This fat mixture could then be substituted for ground fat in a coarse product, such as cotto salami, with minimal change in texture.

Since product stability is not a problem for most U.S. sausage makers (due to the low thermal processing temperatures generally used), it is critical to the future use of fat mixtures that a need for fat mixtures in products be established. The most promising use for fat mixtures is in the production of low fat sausages. Research is needed, however, to optimize fat mixture usage levels in products.

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BIBLIOGRAPHY

Acton, J. C., M. A. Hanna, and L. D. Satterlee. 1981.
    Heat-induced gelation and protein-protein interaction of
    actomyosin. J. Food Biochem. 5:101-103.

Andersson, Y. 1976. Influence of unconventional proteins
    on the texture/consistency properties of meat patties.
    Pages H1:3 - H1:7 in Proceedings of the 22nd European
    meeting of meat research workers. Technical Secretariate,
    Swedish Meat Research Centre, Kavlinge, Sweden.

A.O.A.C. 1970. Official methods of analysis. 11th ed.
    Association of Official Analytical Chemists, Washington,
    D.C.

Aoki, H., O. Taneyama, N. Orimo, and I. Kitigawa. 1981.
    Effect of lipophilization of soy protein in its emulsion
    stabilizing properties. J. Food Sci. 46:1192-1195.

Arima, S., R. Niki, and K. Takase. 1979. Structure of
    β-casein. J. Dairy Res. 46:238-282.

Badley, R. A., D. Atkinson, H. Hauser, D. Oldani, J. P. Green,
    and J. M. Stubbs. 1975. The structure, physical and
    chemical properties of the soybean protein glycinin.
    Biochim. et Biophys. Acta 412:214-228.

Becher, P. 1965. Introduction. Pages 1-3 in Emulsions:
    Theory and practice. 2nd ed. Reinhold Publishing
    Corporation, New York.

Bloomfield, V. A., and R. J. Mead, Jr. 1974. Structure
    and stability of casein micelles. J. Dairy Sci. 58(4):
    592-601.

Borchert, L. L., M. L. Greaser, J. C. Bard, R. G. Cassens, and
    E. J. Briskey. 1967. Electron microscopy of a meat
    emulsion. J. Food Sci. 32:419-421.

Borjedo, J. 1983. Mapping of hydrophobic sites on the surface
    of myosin and its fragments. Biochemistry 22:1182-1187.

Bourne, M. C. 1968. Texture profile of ripening pears. J.
    Food Sci. 33:223-226.

Bourne, M. C. 1974. Principles of objective texture measurement. Page 68 in G. Inglett ed. Texture properties and evaluations of fabricated foods. The AVI Publishing Co., Inc., Westport, Conn.

Bourne, M. C. 1978. Texture profile analysis. Food Technol. 32:68-75.

Boyer, R. A. 1954. High Protein Food Product and Process for Its Preparation. U. S. Patent 2,682,446.

Brady, P. L., and M. E. Huneck. 1985. Correlations of sensory and instrumental evaluations of roast beef texture. J. Food Sci. 50:300-301.

Brennan, J. G., R. Jowitt, and O. A. Mughsi. 1970. Some experiences with the General Foods texturometer. J. Texture Stud. 1:167-184.

Brouwer, F., J. Schut, and J. C. M. Van Zutphen. 1976. The influence of the composition and of some process variables on the properties of liver sausage. Pages E2:3 - E2:6 in Proceedings of the 22nd European meeting of meat research workers. Technical Secretariate, Swedish Meat Research Centre, Kavlinge, Sweden.

Brunner, J. R. 1977. Milk Proteins. Chapter 7 in J. R. Whitaker and S. R. Tannenbaum, eds. Food Proteins. The AVI Publishing Company, Inc., Westport, Conn.

Burge, P. L., and J. C. Acton. 1984. Rheological properties of comminuted meat batters and the relationship to constituent interactions. J. Food Technol. 19:719-724.

Caldironi, H. A., and H. W. Ockerman. 1982. Incorporation of blood proteins into sausages. J. Food Sci. 47:405-408.

Carpenter, J. A., and R. L. Saffle. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29:774-781.

Carroll, R. J., and C. M. Lee. 1981. Meat emulsions-fine structure relationships and stability. Scanning Electron Microscopy 3:105-110.

Catsimpoolas, N., and E. W. Meyer. 1970. Gelation phenomena of soybean globulins: I. Protein-protein interactions. Cereal Chem. 47:559-570.

Catsimpoolas, N., and E. W. Meyer. 1971a. Gelation phenomena of soybean globulins. II. Protein-water miscible solvent interactions. Cereal Chem. 48:150-158.

Catsimpoolas, N., and E. W. Meyer. 1971b. Gelation phenomena of soybean globulins. III. Protein-lipid interactions. Cereal Chem. 48:159-167.

Catsimpoolas, N., D. A. Rogers, S. J. Circle, and E. W. Meyer. 1967. Purification and structural studies of the 11S component of soybean proteins. Cereal Chem. 44:631-637.

Catsimpoolas, N., T. G. Campbell, and E. W. Meyer. 1969. Association-dissociation phenomena of glycinin. Arch. Biochem. Biophys. 313:577-586.

Choi, Y. R., E. W. Lucas, and K. C. Rhee. 1983. Molecular structure and functionalities of protein isolates prepared from defatted cottonseed flour succinylated at various levels. J. Food Sci. 48(4):1275-1278.

Chou, D. H., and C. V. Morr. 1979. Protein-water interactions and functional properties. J. Am. Oil Chem. Soc. 56:53A-61A.

Christian, J. A., and R. L. Saffle. 1967. Plant and animal fats and oils emulsified in a model system with muscle salt-soluble protein. Food Technol. 21:1024-1027.

Christopher, J., E. Pistorius, and B. Axelrod. 1970. Isolation of an isozyme of soybean lipoxygenase. Biochim. Biophys. Acta 198:12-19.

Cichon, R., K. Elkowicz, H. Kozlowska, A. Rutkowski, and W. C. Sauer. 1980. Nutritional evaluation of meat-soya bean protein product blends. J. Sci. Food Agric. 31:677-684.

Circle, S. J., E. W. Meyer, and R. W. Whitney. 1964. Rheology of soy protein dispersions. Effect of heat and other factors on gelation. Cereal Chem. 41:157-172.

Comer, F. W., and S. Dempster. 1981. Functionality of fillers and meat ingredients in comminuted meat products. Can. Inst. Food Sci. Technol. J. 14(4):295-303.

Comer, F. W., N. Chew, L. Lovelock, and P. Allan-Wojtas. 1986. Comminuted meat products: Functional and micro-structural effects of fillers and meat ingredients. Can. Inst. Food Sci. Technol. J. 19:68-74.

Crenwelge, D. D., C. W. Dill, P. T. Tybor, and W. A. Landmann. 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci. 39:175-177.

Damodaran, S., and J. E. Kinsella. 1982. Effect of - conglycinin on thermal aggregation of glycinin. J. Agric. Food Chem. 30:812-817.

Deshpande, S. S., P. D. Ragnekar, S. K. Sathe, and D. K. Salunkhe. 1983. Functional properties of wheat-bean composite flours. J. Food Sci. 48:1659-1662.

Ehninger, J. N., and D. E. Pratt. 1974. Some factors influencing gelation and stability of soy protein dispersions. J. Food Sci. 39:892-896.

El-Negoumy, A. M. 1978. Changes in sodium caseinate heated in various model systems. J. Dairy Sci. 61:20-27.

Erdman, J. W. 1979. Oil-seed phytates: Nutritional implications. J. Am. Oil Chem. Soc. 56:736-741.

Ferry, J. D. 1948. Protein gels. Adv. Protein Chem. 4:1-78.

Fleming, S. E., and F. W. Sosulski. 1975. Gelation and thickening phenomena of vegetable protein products. J. Food Sci. 40:805-807.

Flint, F. O., and R. F. P. Johnson. 1981. A study of film formation by soy protein isolate. J. Food Sci. 46:1351-1353.

Foegeding, E. A., C. E. Allen, and W. R. Dayton. 1983. Thermally-induced gelation and interactions of myosin, albumin and fibrinogen. Proc. 36th Ann. Rec. Meats Conf. 35:190-198.

Fox, J. B., R. K. Jenkins, and S. A. Ackerman. 1983. Texture of emulsified cooked meat products by three different methods of measurement. J. Food Sci. 48: 1025-1030.

Franzen, K., and J. Kinsella. 1975. Physiochemical aspects of food flavoring. Chem. Ind. pp. 505-508.

Friberg, S. E., and M. El-Nokaly. 1983. Multilayer emulsions. Chap. 5 in M. Peleg and E. N. B. Bagley, eds. Physical Properties of Foods. The AVI Publishing Co., Inc., Westport, Conn.

Fukazawa, T., Y. Hashimoto, and T. Yasui. 1961. Effect of some proteins on the binding quality of an experimental sausage. J. Food Sci. 26:541-549.

Furukawa, T., S. Ohta, and A. Yamamoto. 1979. Texture-structure relationships in heat-induced soy protein gels. J. Texture Stud. 10:333-346.

Furukawa, T., and S. Ohta. 1982. Mechanical and water-holding properties of heat-induced soy protein gels as related to their structural aspects. J. Texture Stud. 13:59-69.

Gallimore, W. W. 1976. Estimated sale and impact of soy-beef blends in grocery stores. Nat. Food Sit. 155:37-44.

Galluzzo, S. J., and J. M. Regenstein. 1978. Emulsion capacity and timed emulsification of chicken breast muscle myosin. J. Food Sci. 43:1757-1761.

German, B., S. Damodaran, and J. E. Kinsella. 1982. Thermal dissociation and association behavior of soy proteins. J. Agric. Food Chem. 30:807-811.

Gillett, T. A., D. E. Meiburg, C. L. Brown, and S. Simon. 1977. Parameters affecting meat protein extraction and interpretation of model system data for meat emulsion formation. J. Food Sci. 42:1606-1609.

Haga, S., and T. Ohashi. 1984. Heat induced gelation of a mixture of myosin B and soybean protein. Agric. Biol. Chem. 48:1001-1007.

Hamm, R. 1970. Properties of meat proteins. Pages 167-185 in R. Lawrie, ed. Proteins as Human Food. The AVI Publishing Co., Inc., Westport, Conn.

Hand, L. R., R. N. Terrell, and G. C. Smith. 1983. Effects of non-meat protein products on properties of fat-batters and mortadella sausage. J. Food Sci. 48:119-124.

Hansen, L. F. 1960. Emulsion formation in a finely comminuted sausage. Food Technol. 14:565-571.

Hashizume, K., and K. Watanabe. 1979. Influence of heating temperature and conformational changes of soybean proteins. Agric. Biol. Chem. 43:681-690.

Hayes, J. F., P. M. Southby, and L. L. Muller. 1968. Factors affecting the viscosity of caseinates in dispersions of high concentrations. J. Dairy Res. 35:31-47.

Hegarty, G. R., L. J. Bratzler, and A. M. Pearson. 1963. Studies on the emulsifying properties of some intra-cellular beef muscle proteins. J. Food Sci. 28:663-668.

Helmer, R. L., and R. L. Saffle. 1963. Effect of chopping temperature on the stability of sausage emulsions. Food Technol. 17:115-119.

Helwig, J. T., and K. A. Council. 1979. Sas User's Guide, SAS Institute Inc., Raleigh, N. C.

Hermansson, A. M. 1972. Functional properties of proteins for foods: Swelling. Lebensm.-Wiss. Technol. 5:24-29.

Hermansson, A. M. 1975. Functional properties of added proteins correlated with properties of meat systems. Effect on texture of a meat product. J. Food Sci. 40:611-614.

Hermansson, A. M. 1978a. Physico-chemical aspects of soy proteins structure formation. J. Texture Stud. 9:33-58.

Hermansson, A. M. 1978b. The effect of some functional properties of added proteins on properties of meat systems. Pages H3.3 - H3.7 in Proceedings of the 24th European Meeting of Meat Research Workers. Bundesanstalt fur Fleischforschung, Kulmbach Bundesrepublik, Deutschland.

Hermansson, A. M. 1979. Methods of studying functional characteristics of vegetable proteins. J. Am. Oil Chem. Soc. 56:272-279.

Hermansson, A. M. 1985. Soy protein gelation. J. Am. Oil Chem. Soc. 63:658-666.

Hermansson, A. M., and C. Akesson. 1975. Functional properties of added proteins correlated with properties of meat systems. Effect of salt on water binding properties of model meat systems. J. Food Sci. 40:603-610.

Hiratsuki, M., H. Taniguchi, H. Narita, and M. Kito. 1984. Increase in emulsification activity of soy lecithin-soy protein complex by ethanol and heat treatments. J. Food Sci. 49:1105-1110.

Hoogenkamp, H. 1979. Practical applications of milk proteins in meat products. De Melkindustrie, Veghel, Netherlands.

Hoogenkamp, H. 1985. Super emulsions. Meat Processing 24(2):32-36.

Horan, F. E. 1974. Meat Analogs. Chapter 9 in A. Altschul, ed. New Protein Foods. Academic Press, Inc., New York, N.Y.

Hoshi, Y. and F. Yamauchi. 1983. Determination of sulfhydryl and disulfide contents of soybean 11S globulin and their change in lyophilization. Agric. Biol. Chem. 47:2217-2222.

Hoshi, Y., F. Yamauchi, and K. Shibasaki. 1982. Effect of relative humidity on aggregation of soybean 7S and 11S globulins. Agric. Biol. Chem. 46(6):1513-1517.

Hutton, C. W., and A. M. Campbell. 1977. Functional properties of a soy concentrate and a soy isolate in simple systems and in a food system. Emulsion properties, thickening function and fat absorption. J. Food Sci. 42:457-460.

Inklaar, P. A., and J. Fortuin. 1969. Determining the emulsifying and emulsion stabilizing capacity of protein meat additives. Food Technol. 23:103-107.

Ishioroshi, M., K. Samejima, Y. Aria, and T. Yasui. 1980. Effect of blocking the myosin-actin interaction in heat-induced gelation of myosin in the presence of actin. Agric. Biol. Chem. 44:2185-2194.

Jolles, J., F. Schoentgen, C. Alais, and P. Jolles. 1972. Studies on the primary structure of cow κ-casein: The primary structure of cow para-κ-casein. Chimia 26:645-646.

Jones, K. W., and R. W. Mandigo. 1982. Effects of chopping temperature on the microstructure of meat emulsions. J. Food Sci. 47:1930-1935.

Jones, K. W. 1984. Protein lipid interactions in processed meats. Proceedings of the 37th Ann. Rec. Meat Conf. National Livestock and Meat Board. Chicago, Ill.

Jones, R. T. 1964. The structure of proteins. Page 34 <u>in</u> H. W. Schultz and A. F. Anglemeir, eds. Proteins and Their Reactions. The AVI Publishing Co., Inc., Westport, Conn.

Jongsma, J. 1982. New technologies for the preparation of milk protein/fat/water emulsions. Proceedings Lecture at the Norwegian Meat Processing Seminar. De Melkindustrie, Veghel, Netherlands.

Kamat, V. B., G. E. Graham, and M. A. F. Davis. 1978. Vegetable protein: Lipid interactions. Cereal Chem. 55:295-307.

Kato, A., and S. Nakai. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochim. Biophys. Acta 624:13-21.

Katsaras, K. and R. Stenzel. 1984. The micro-structure of frankfurter-type sausages as observed under the scanning and the transmission electron microscope. Fleischwirtsch. 64:951-952.

Keeton, J. T., E. A. Foegeding, and C. Patana-Anake. 1983. A comparison of nonmeat proteins, sodium tripolyphosphate and processing temperature effects in physical and sensory properties of frankfurters. J. Food Sci. 49: 1462-1465,1474.

Keshavarz, E., and S. Nakai. 1979. The relationship between hydrophobicity and interfacial tension of proteins. Biochim. Biophys. Acta 576:269-279.

Kinsella, J. E. 1976. Functional properties of proteins in foods: A survey. CRC Crit. Rev. in Food Sci. Nutr. 7:219-280.

Kinsella, J. E. 1979. Functional properties of soy proteins. J. Am. Oil Chem. Soc. 56:242-258.

Kinsella, J. E. 1984. Milk proteins: physicochemical and functional properties. Pages 197-262 in T. Furia ed. CRC Critical reviews in food science and nutrition. CRC Press, Boca Raton, FL.

Kitamura, K., T. Takagi, and K. Shibasaki. 1976. Subunit structure soybean 11S globulin. Agric. Biol. Chem. 40(9):1837-1844.

Koshiyama, I. 1970. Dissociation into subunits of a 7S protein in soybean globulines with urea and sodium dodecyl sulfate. Agric. Biol. Chem. 34:1815-1820.

LaBudde, R. A., and G. C. Selfridge. 1982. Make it better with preblending. Pages 43-48 in Proc. 3rd Ann. Proc. Meats Short Course. Iowa State Univ., Ames, Ia.

Labuza, T. P., and G. C. Busk. 1979. An analysis of the water binding in gels. J. Food Sci. 44:1379-1385.

Larkins, B. A. 1981. Seed storage proteins: Characterization and biosynthesis. Chapter 11 in A. Marcus ed. Biochemistry of plants: Proteins and nucleic acids. Academic Press, Inc., London.

Lauck, R. M. 1975. The functionality of binders in meat emulsions. J. Food Sci. 40:736-740.

Lee, C. M., R. J. Carroll, and A. Abdollah. 1981. A microscopical study of the structure of meat emulsions and its relationship to thermal stability. J. Food Sci. 46:1789-1793, 1804.

Lin, L. C., and T. Ito. 1985. Rheological property of myosin B fibre and the fibre of the mixture of myosin B and soya protein; and the interaction between myosin B and soya protein. J. Food Technol. 20:219-235.

Lis, H., C. Fridman, N. Sharon, and E. Katchalski. 1966. Multiple hemagglutivins in Soybeans. Arch. Biochem. Biophys. 117:301-309.

MacLean-Fletcher, S., and T. D. Pollard. 1980. Viscometric analysis of the gelation of Acanthamoebe extracts and purification of two gelation factors. J. Cell Biol. 85:414-418.

Marshall, W. H., T. R. Dutson, Z. L. Carpenter, and G. C. Smith. 1975. A simple method for emulsion end-point determinations. J. Food Sci. 40:896-897.

Mattil, K. F. 1971. The functional requirements of proteins for foods. J. Am. Oil Chem. Soc. 48:477-480.

McKenzie, H. A. 1967. Milk proteins. Adv. Protein Chem. 22:55-234.

McWatters, K. H. 1977. Performance of defatted peanut, soybean and field pea meals as extenders in ground beef patties. J. Food Sci. 42:1492-1495.

McWatters, K. H., and J. P. Cherry. 1977. Emulsification, foaming and protein solubility properties of defatted soybean, peanut, field pea and pecan flours. J. Food Sci. 42:1444-1447, 1450.

McWatters, K. H., and J. P. Cherry. 1981. Emulsification: vegetable proteins. Chapter 11 in J. P. Cherry, ed. Protein functionality in foods. Am. Chem. Soc., Washington, D.C.

Mercier, J. C., G. Brignon, et B. Ribadeau-Dumas. 1973. Structure primarie de la caseine κβ bovine (in French, English summary). Eur. J. Biochem. 35:222-235.

Meyer, A. J., W. L. Brown, N. E. Giltner, and J. R. Guinn. 1964. Effect of emulsifiers on the stability of sausage emulsions. Food Technol. 18:1796-1798.

Mita, T., K. Yamada, S. Matsumoto, and D. Yonezawa. 1973. Dispersion state of protein-stabilized emulsions. J. Texture Stud. 4:41-52.

Miura, M. and F. Yamauchi. 1983. Effects of lipids on the rheological properties of aqueous soybean protein dispersions. Agric. Biol. Chem. 47:2217-2222.

Miura, T., K. Yamada, S. Matsumoto, and D. Yonezawa. 1983. Effects of lipids on the rheological properties of aqueous soybean protein dispersions. Agric. Biol. Chem. 47:2217-2222.

Moreira, M. A., M. A. Hermodson, B. A. Larkins, and N. C. Nielsen. 1979. Partial characerization of the acidic and basic polypeptides of glycinin. J. Biol. Chem. 254:9921-9926.

Mori, T., T. Nakamura, and S. Utsumi. 1981. Gelation mechanism of soybean 11S globulin: Formation of soluble aggregates as transient intermediates. J. Food Sci. 46:26-30.

Morr, C. V. 1979a. Utilization of milk proteins as starting materials for other food stuffs. J. Dairy Res. 46: 369-376.

Morr, C. V. 1979b. Symposium: milk proteins in dairy and food processing. Chemistry of milk proteins in food processing. J. Dairy Sci. 58:977-984.

Morr, C. V. 1979c. Conformation and functionality of milk proteins. Pages 65-79 in Advances in Chemistry Series No. 92. Am. Chem. Soc., Washington, D. C.

Morr, C. V. 1981. Emulsifiers: milk proteins. Chapter 10 in J. P. Cherry, ed. Protein functionality in foods. Am. Chem. Soc., Washington, D. C.

Muller, L. L. 1971. Manufacture and uses of casein and coprecipitate. Dairy Sci. Abstr. 33:659-674.

Nakamura, T., S. Utsumi, and T. Mori. 1984. Network structure formation in thermally induced gelation of glycinin. J. Agric. Food Chem. 32:349-352.

Nakayama, T., and Y. Sato. 1971. Relationships between binding quality of meat and myofibrillar proteins. III. Contribution of Myosin A and actin to rheological properties of heated minced-meat gel. J. Texture Stud. 2:75-88.

Ono, T., K. Yutani, and S. Odagiri. 1974a. The secondary structure of αs-casein. Agric. Biol. Chem. 38:1603-1608.

Ono, T., K. Yutani, and S. Odagiri. 1974b. Conformation of αs-casein in various concentrations. Agric. Biol. Chem. 38:1609-1616.

Oosawa, F., S. Asakura, K. Hotta, N. Imai, and T. Ooi. 1959. G-F transformation of actin as a fibrous condensation. J. Polym. Sci. 37:323-328

Ozimek, G., and S. Poznanski. 1981. Influence of an addition of textured milk proteins upon physico-chemical properties of meat mixtures. J. Food Sci. 47:234-239, 245.

Pearce, K. N., and J. E. Kinsella. 1978. Emulsifying properties of proteins: Evaluation of turbidimetric technique. J. Agric. Food Chem. 26:716-723.

Pearson, A. M., M. E. Spooner, G. R. Hegarty, and L. J. Bratzler. 1965. The emulsifying capacity and stability of soy sodium proteinate, potassium caseinate and nonfat dry milk. Food Technol. 19:1841-1845.

Pearson, J. T., and A. E. Alexander. 1968. The application of monolayer techniques to a study of protein-surfactant interaction. J. Colloid Interface Sci. 27:53-61.

Peng, I. C., W. R. Dayton, D. W. Quass, and C. E. Allen 1982a. Investigations of soybean 11S protein and myosin interaction by solubility, turbidity and titration studies. J. Food Sci. 47:1976-1983.

Peng, I. C., W. R. Dayton, D. W. Quass, and C. E. Allen. 1982b. Studies on the subunits involved in the interaction of soybean 11S protein and myosin. J. Food Sci. 47:1984-1994.

Petrowski, G. E. 1979. Emulsion stability and its relation to foods. Adv. Food Res. 22:310-359.

Phillips, M. C. 1981. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. Food Technol. 35:50-51, 54-57.

Pour-El, A. 1981. Protein functionality: classification, definition and methodology. Chapter 1 in J. P. Cherry, ed. Protein functionality in foods. Am. Chem. Soc., Washington, D.C.

Puski, G. 1976. A review of methodology for emulsification properties of plant proteins. Cereal Chem. 53: 650-655.

Rackis, J. J., J. E. McGhee, D. H. Honig, and A. N. Booth. 1975. Processing soybeans into foods: selected aspects of nutrition and flavor. J. Am. Oil Chem. Soc. 52:249A-253A.

Rakosky, J., Jr. 1970. Soy products for the meat industry. J. Agric. Food Chem. 18:1005-1009.

Regenstein, J. M., and C. E. Regenstein. 1984. Protein functionality for food scientists. Pages 306-309 in Food protein chemistry. Academic Press, Inc., Orlando, FL.

Rey, D. K., and T. P. Labuza. 1981. Characterization of the effect of solutes on the water-binding and gel strength properties of carrageenan. J. Food Sci. 46:786-789, 793.

Ribadeau-Dumas, B., G. Brignon, F. Gorsclaude, et J. C. Mercier. 1972. Structure primarie de la caseine β bovine. (in French, English summary). Eur. J. Biochem. 25:505-514.

Rivero de Padua, M. 1983. Some functional and utilization characteristics of sesame flour and proteins. J. Food Sci. 48:1145-1147.

Rock, H., E. F. Sipos, and E. W. Meyer. 1966. Soy protein-its role in processed meat production. Meat 32:52-56.

Rongey, E. H. 1965. A simple objective test for sausage emulsion quality. Pages 99-106 in Proc. Meat Ind. Res. Conf., Amer. Meat Inst. Found., Arlington, VA.

Sabharwal, K., and D. G. Vakaleris. 1972. Stability of fluid food emulsions. I. Effects of emulsifiers, electrolytes and sodium caseinate. J. Dairy Sci. 55:277-282.

Saffle, R. L. 1968. Meat emulsions. Adv. Food Res. 16:105-160.

Saio, K., and T. Watanabe. 1978. Differences in functional properties of 7S and 11S soybean proteins. J. Texture Stud. 9:135-157.

Samejima, K., Y. Hashimo, T. Yasui, and T. Fukaza. 1969. Heat gelling properties of myosin, actin, actomyosin and myosin subunits in a saline model system. J. Food Sci. 34:242-245.

Samejima, K., M. Ishioroshi, and T. Yasui. 1981. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. J. Food Sci. 46:1412-1416.

Samejima, K., M. Ishioroshi, and T. Yasui. 1982. Heat-induced gelling properties of actomyosin: Effect of tropomyosin and troponin. Agric. Biol. Chem. 46:535-540.

Scheraga, H. A. 1963. Intramolecular bonds in proteins. II. Noncovalent bonds. In H. Neurath, ed. The proteins. Academic Press, Inc., New York.

Schmidt, G. R., R. F. Mawson, and D. G. Siegel. 1981. Functionality of a protein matrix in comminuted meat products. Food Technol. 35:235-237,252.

Schmidt, G. R., W. J. Means, D. F. Herriot, and B. F. Miller. 1982. The functionality of soy protein concentrate in canned luncheon meat. Pages 302-305 in Proceedings of the 28th European Meeting of Meat Research Workers, Madrid, Spain.

Schut, J. 1976. Meat Emulsions. Chapter 8 in S. Friberg, ed. Food emulsions. Marcel Dekker, Inc., New York, NY.

Schweid, J. and R. T. Toledo. 1981. Changes in physical properties of meat batters during heating. J. Food Sci. 46:850-855,859.

Seman, D. L., D. G. Olson, and R. W. Mandigo. 1980. Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J. Food Sci. 45:1116-1121.

Shibasaki, K., K. Okubo, and T. Ono. 1969. Food chemical studies of soybean proteins. V. On the insoluble protein components of defatted soybean heated by steaming. J. Food Sci. Technol. Japan 16:22-26.

Shimada, K., and S. Matsushita. 1981a. Effects of oils on thermal gelation of soybean protein. Agric. Biol. Chem. 45:2877-2881.

Shimada, K., and S. Matsushita. 1981b. Effects of salts and denaturants on thermocoagulation of proteins. J. Agric. Food Chem. 29:15-20.

Shimizu, M., T. Takahashi, S. Kaminogawa, and K. Yamauchi. 1983. Adsorption onto an oil surface and emulsifying properties of bovine αs1-casein in relation to its molecular structure. J. Agric. Food Chem. 31:1214-1218.

Siegel, D. G., K. E. Church, and G. R. Schmidt. 1979. Gel structure of nonmeat proteins as related to their ability to bind meat pieces. J. Food Sci. 44:1276-1279.

Smith, G. C., H. Juhn, S. L. Carpenter, K. F. Mattil, and C. M. Cater. 1973. Efficacy of protein additives as emulsion stabilizers in frankfurters. J. Food Sci. 38:849-855.

Sofos, J. N. 1983. Effects of reduced salt (NaCl) levels on sensory and instrumental evaluation of frankfurters. J. Food Sci. 48:1692-1695,1699.

Sofos, J. N., and C. E. Allen. 1977. Effects of lean meat source and levels of fat and soy protein on the properties of weiner-type products. J. Food Sci. 42:875-877.

Sofos, J. N., I. Noda, and C. E. Allen. 1977. Effects of soy proteins and their levels of incorporation on the properties of weiner-type products. J. Food Sci. 42:879-884.

Stainsby, G. 1977. The gelation gel and sol-gel transformation. Pages 179-185 in A. G. Ward and A. Courts, eds. The science and technology of gelation. Academic Press, New York, NY.

Stutz, E. L., A. J. DelVecchio, and R. J. Tenney. 1973. The role of emulsifiers and dough conditioners in foods. Food Prod. Devel. 7:52-54,56,58,60.

Swaisgood, H. E., and J. R. Brunner. 1963. Characteristics of kappa-casein in the presence of various dissociating agents. Biochem. Biophys. Res. Commun. 12:148-151.

Swift, C. E., C. Lockett, and A. J. Fryar. 1961. Comminuted meat emulsions-the capacity of meat for emulsifying fat. Food Technol. 15:468-473.

Tachibana, I., and K. Inokuchi. 1953. Rheological approach for the study of protein monolayers. J. Colloid Sci. 8:341-347

Tachibana, T., K. Inokuchi, and T. Inokuchi. 1957. Surface gelation of ovalbumin monolayers at the air-water interface. Biochim. Biophys. Acta 24:174-179.

Tanford, C. 1968. Protein denaturation. Adv. Protein Chem. 23:121-135.

Tantikarnjathep, K. 1980. Vacuum emulsification of meat. Ph.D. Thesis. Iowa State University, Ames, Ia.

Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L.Dugam, Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44-48.

Terrell, R. N., C. H. Crenwelge, T. R. Dutson, and G. C. Smith. 1982. A technique to measure binding properties of non-meat proteins in muscle-junction formation. J. Food Sci. 47:711-713.

Thanh, V. H., and K. Shibasaki. 1978a. Major proteins of soybean seeds. Subunit structure of β-conglycinon. J. Agric. Food Chem. 26:692-695.

Thanh, V. H., and K. Shibasaki. 1978b. Major proteins of soybean seeds. Reconstitution of β-conglycinon from its subunits. J. Agric. Food Chem. 26:695-698.

Titus, T. C., N. N. Wiancko, H. F. Barbour, and J. B. Mickle. 1968. Emulsifier efficiency in model systems of milk fat or soybean oil and water. Food Technol. 22:1449-1451.

Tombs, M. P. 1969. Protein bodies of the the soybean. Plant Physiol. 42:797-813.

Towler, C., L. K. Creamer, and C. R. Southward. 1981. The effect of disulphide-bond reducing agents on the viscosity of casein solutions. New Zealand J. Dairy Sci. Technol. 16:155-165.

Townsend, W. E., L. P. Witnauer, J. A. Riloff, and C. E. Swift. 1968. Comminuted meat emulsions: Differential thermal analysis of fat transitions. Food Technol. 22:319-338.

Trumbetas, J., J. A. Fioriti, and R. J. Sims. 1976. Applications of pulsed NMR to fatty emulsions. J. Am. Oil Chem. Soc. 52:722-726.

Tsai, R., R. G. Cassens, and E. J. Briskey. 1972. The emulsifying properties of purified muscle proteins. J. Food Sci. 37:286-288.

Tsugo, T., and K. Yamauchi. 1956. On the break-up of casein by rennin and its relationship to milk coagulation. XIV Int. Dairy Congr. 2:588-595.

Tung, M. A. 1978. Rheology of protein dispersions. J. Texture Stud. 9:3-31.

Umeya, J., F. Yamauchi, and K. Shibasaki. 1980. Hardening and softening properties of soybean, protein - water suspending systems. Agric. Biol. Chem. 44:1321-1326.

U.S.D.A. 1973. Meat and poultry inspection regulation 318.7 (c) (4) in Meat and poultry inspection regulations, Meat and poultry inspection program, Animal and Plant Health Inspection Service, United States Department of Agriculture, Washington, D.C., May 1973.

Utsumi, S., T. Nakamura, and T. Mori. 1982. A micro-method for the measurement of gel properties of soybean 11S globulin. Agric. Biol. Chem. 46:1923-1924.

Vananuvat, P., and J. E. Kinsella. 1975. Some functional properties of protein isolates from yeast, Saccharomyces fragilis. J. Agric. Food Chem. 23:613-616.

Van den Oord, A. H. A., and P. R. Visser. 1973. State and distribution of fat in finely comminuted meat products (frankfurter-type sausage). Fleischwirtschaft 10:1427-1432.

Visser, F. M. W. 1980. New information on meat emulsion technology. Ailmentaria 80. Barcelona 3/11-12/1980. De Melkindustrie, Veghel, Netherlands.

Visser, F. M. W. 1983a. The stability of finely comminuted meat products and the role of milk protein in it. Crown Mills Seminars for the South African Meat Industry. De Melkindustrie, Veghel, Netherlands.

Visser, F. M. W. 1983b. Preparing caseinate-fat-water emulsions. Crown Mills Seminars for the South African Meat Industry. De Melkindustrie, Veghel, Netherlands.

Volkert, M. A., and B. P. Klein. 1978. Protein dispersability and emulsion characteristics of four soy products. J. Food Sci. 44:93-96.

Von Stratum, P. G., and M. Rudrum. 1979. Effects of consumption of processed soy proteins on minerals and digestion in man. J. Am. Oil Chem. Soc. 56:130-134.

Voutsinas, L. P., E. Cheung, and S. Nakai. 1983. Relationships of hydrophobicity to emulsifying properties of heat-denatured proteins. J. Food Sci. 48:26-32.

Wake, R. G. 1959. Studies of casein. V. The action of
    rennin on casein. Aust. J. Biol. Sci. 12:479-489.

Walstra, P. 1984. Some properties of emulsions in relation
    to processing. Pages 64-68 in B. Krol, P. S. van Roon,
    and J. H. Houben, eds. Trends in modern meat technology:
    Proceedings of the International Symposium. Pudoc, Center
    for Agricultural Publishing and Documentation,
    Wageningen, Netherlands.

Webb, N. B., F. J. Ivey, H. B. Craig, V. A. Jones, and R. J.
    Monroe. 1970. The measurement of emulsifying capacity
    by electrical resistance. J. Food Sci. 35:501-504.

Welch, R. M., and D. R. VanCampen. 1975. Iron availability
    to rats from soybeans. J. Nutr. 105:253-256.

Welsby, D., M. McCarthy, and D. Doolan. 1982. The use of
    milk proteins as hydrocolloids in food systems. Prog.
    Fd. Nutr. Sci. 6:221-231.

Wierbicki, E. V., R. Cahill, and F. E. Deatherage. 1957.
    Changes in the water-holding capacity and cationic
    shifts during the heating and freezing and thawing of
    meat as revealed by a simple centrifugal method of
    measuring shrinkage. Food Technol. 11:69-74.

Wolf, W. J. 1970. Soybean proteins: Their functional,
    chemical and physical properties. J. Agric. Food Chem.
    18:969-976.

Wolf, W. J. 1972. Purification and properties of proteins.
    Pages 93-143 in A. K. Smith and S. J. Circle, eds.
    Soybeans: chemistry and technology. The AVI Publishing
    Co., Inc., Westport, Conn.

Wolf, W. J. 1977. Soy proteins for fabricated foods.
    Chapter 7 in Fabricated foods. The AVI Publishing Co.,
    Inc., Westport, Conn.

Wolf, W. J., and D. R. Briggs. 1959. Purification and
    characterization of the 11S component of soybean
    proteins. Arch. Biochem. Biophys. 85:186-199.

Wolf, W. J., and J. C. Cowan. 1975. Functional properties
    of soybean proteins. Table 28 in Soybeans as a food
    source, 2nd ed., CRC Press, Inc., Cleveland,
    OH.

Wolf, W. J., and T. Tamura. 1969. Heat denaturation of
    soybean 11S protein. Cer Chem. 46:331-334.

Wolford, K. M. 1973. Beef/soy: Consumer acceptance. General Mills Communication. Minneapolis, MN. Nov. 13, 1973.

Yamagishi, F., F. Yamauchi, and K. Shibasaki. 1981. Electrophoretical and differential thermal analysis of soybean 11S globulin heated in the presence of N-ethylmaleimide. Agric. Biol. Chem. 45:1661-1668.

Yamagishi, F., F. Ebina, and F. Yamauchi. 1982. Analysis of the state of aromatic amino acid residues in heated soybean 7S globulin by absorption derivative spectrophotometry and spectrofluorimetry. Agric. Biol. Chem. 46:2441-2448.

Yamagishi, F., A. Miyakawa, N. Nada, and F. Yamauchi. 1983. Isolation and electrophoretic analysis of heat-induced products of mixed soybean 7S and 11S globulins. Agric. Biol. Chem. 47:1229-1237.

Yamauchi, F., Y. Ogawa, Y. Kamata, and K. Shibasaki. 1982. Emulsifying properties of soybean β-conglycinin: Evaluation by turbidimetry. Agric. Biol. Chem. 46:615-621.

Yasui, T., and K. Ishioroshi. 1980. Heat-induced gelation in the presence of actin. J. Food Biochem. 4:61-78.

Yasui, T., M. Ishioroshi, and K. Samejima. 1982. Effect of actomyosin on heat-induced gelation of myosin. Agric. Biol. Chem. 46:1049-1059.

Yasumatsu, K., J. Toda, T. Wada, M. Misaka, and K. Ishii. 1972. Studies on the functional properties of food-grade soybean products. Part III. Properties of heat-coagulated gels from soybean products. Agric. Biol. Chem. 36:537-543.

Young, V. R., and J. Janghorbani. 1981. Soy proteins in human diets in relation to bioavailability of iron and zinc: A brief overview. Cereal Chem. 58:12-18.

Young, V. R., N. S. Scrimshaw, B. Torun and F. Viteri. 1979. Soybean protein in human nutrition: An overview. J. Am. Oil Chem. Soc. 56:110-120.

Zayas, J. F. 1985. Structural and water binding properties of meat emulsions prepared with emulsified and unemulsified fat. J. Food Sci. 50(3):688-692.

Ziegler, G. R., and J. C. Acton. 1984. Mechanisms of gel formation by proteins of muscle tissue. Food Technol. 38:67-72.