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FUNCTIONAL PROPERTIES OF MODIFIED OILSEED PROTEIN CONCENTRATES AND ISOLATES

by

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ABSTRACT

Compositional, structural and functional properties of nine oilseed concentrates and isolates were evaluated and compared. Three product sources, canola (rapeseed), sunflower and soybean were investigated, each treated during processing by enzyme-hydrolysis or linoleic acid addition, or left untreated as controls.

Protein, carbohydrate and moisture contents of the products were measured, and examination of the gross structure of 10% aqueous dispersions was carried out using light microscopy techniques. Several functionality tests were performed, including determinations of water holding and water hydration capacities, protein solubility, steady shear flow behavior and viscoelasticity. Scanning electron microscopy was used to examine the microstructure of the gel-like materials produced when 10% aqueous dispersions of the products were heated in a boiling water bath.

Both trypsin and linoleate treatment were observed to significantly affect the structural and functional properties of the products, although the treatment effects differed among sources in some cases. In general, trypsin-treated products contained smaller particles in dispersion, formed weaker gels on heating and exhibited higher solubility and water hydration capacities, and lower apparent viscosities in 10% dispersion. A trend toward larger particles in linoleate-treated product dispersions was observed along with stronger gel-forming abilities, increased solubilities, water hydration capacities and apparent viscosities in 10% dispersion.

Simple correlation coefficients between several measured functional and compositional variables were computed to identify relationships among the physico-chemical properties. Both the size of the dispersed phase particles and the degree of interaction of the protein and carbohydrate components with water were determined to be important in governing functional properties.

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INTRODUCTION

Oilseeds have become important agricultural crops throughout the world. Although grown primarily as a source of oil, considerable interest has been focussed recently on the protein fractions of these commodities. Following removal of the oils by pressing and solvent extraction, the defatted meals may contain over 50% protein. The meals are extensively utilized in animal feeds, while a small proportion is incorporated into foods for human consumption. Processing of the meals to remove the solvent and varying amounts of soluble and insoluble carbohydrate must be carried out on products destined for use in foods. The end product may contain 50 to 95% protein, depending on the processing techniques employed.

Soybean ranks first in world oilseed production followed by cottonseed, peanut, sunflower and rapeseed (canola) (FAO, 1978). Of these, only canola and sunflower can be produced in large quantities in Canada.

The nutritional value of these high-protein oilseed products is high, with Protein Efficiency Ratios ranging as high as 1.8 (Wolf and Cowan, 1971). However, often it is the functional and not the nutritional qualities that determine the acceptability of the product to the consumer. Important functional properties are water hydration, emulsification, viscosity, gel formation, foaming and solubility. Evaluations of these properties provide indications of the structural and textural qualities of foods into which the oilseed products may be incorporated.

Improvements in functionality of an oilseed protein product may be achieved by manipulating processing conditions. High protein solubility can be attained

by maintaining low temperatures. The addition of modifying agents, such as enzymes or surfactants, is another method of controlling functionality.

The research described in this thesis was undertaken to investigate the effects of enzymatic and fatty acid treatment on the functional properties of soybean and sunflower concentrates and a canola (rapeseed) isolate. Several functional properties were evaluated including protein solubility, water hydration capacity and flow behavior. The composition of the products and their structural properties were also investigated.

LITERATURE REVIEW

A. General

The production and processing of oilseed crops has expanded over the past 40 years to such an extent that oilseeds now rank among the most important agricultural cash crops throughout the world. Soybean is the most dominant oilseed on the world scene. Total world production of this crop in 1978 amounted to over 80 million metric tonnes (FAO, 1978). Although the oil content is relatively low, the crop is easily cultivated and provides a high crop yield (Seal, 1978). Processing of soybeans to separate the oil from the meal fractions began in the 1930's. The resulting oil is used for salad and cooking oils, and is hydrogenated to produce margarines and shortenings.

The defatted meal is used predominantly as a protein source for animal feeds, however a small but growing proportion (3-4%) (Kinsella, 1979) is being used as a food ingredient. For food uses, the defatted meal is further processed into three classes of protein products. Soy flours are defatted, desolventized, deodorized and ground flakes which are used extensively in bakery and cereal products. Concentrates are prepared by removing soluble components from defatted soy flour using acidic, aqueous ethanol or hot water leaching agents. These products have better flavor and color and are higher in protein than the flours and can therefore be used in greater quantities in many of the same foods. Soy isolates are produced from flour that has been subjected to a minimum of heat treatment. The protein is dissolved in dilute alkali (pH 8.0), insolubles are removed by centrifugation or filtration and the protein is precipitated out by lowering the pH to the isoelectric range (near pH 4.5). Isolates are used in the manufacture of comminuted meats and

dairy foods where good emulsifying, thickening and gelling properties are important (Wolf and Cowan, 1971).

Soybean proteins are mainly globulins, ranging in molecular weight from 8,000 to 600,000 daltons, with minimum solubilities near pH 4.5. The major globulins are described by their sedimentation coefficients, 7 S and 11 S, and have complex quaternary structures (Wolf, 1970). The 7 S fraction contains at least four different proteins, and has a carbohydrate content of approximately 6% (Wolf, 1977). Soy proteins are relatively high in the amino acid lysine, as compared to other cereals; however, they are low in methionine.

Other oilseed crops have been processed to produce protein-rich products similar to soy flours, concentrates and isolates. Two oilseeds which are suitable for cultivation in Canadian agricultural and climatic conditions are rapeseed (canola) and sunflower.

Sunflower ranks fourth in importance among world vegetable oil crops, and the defatted meal has considerable potential as a food protein source. Although deficient in lysine, the protein is highly digestible and contains no known toxic or anti-nutritive factors. Sosulski et al. (1973) devised a procedure for extracting low molecular weight compounds that were found to be responsible for previously reported disagreeable dark brown and green colors.

Globulins constitute 70 to 79% of the proteins from sunflower. The major globulin has a sedimentation coefficient of 12.1 S and a molecular weight of about 330,000 daltons. The next most predominant globulin has a molecular weight of 20,000 daltons (Sabir et al., 1973).

Rapeseed ranks fifth in total world oilseed production. Food uses of the meal have been limited until recently, due to high fiber levels and the presence of glucosinolates which have antithyroid activity. Low glucosinolate

varieties of rapeseed, safe for human consumption, are now being grown in Canada and these new varieties are called canola, rather than rapeseed, to distinguish them from earlier varieties (Biely, 1980).

A high molecular weight globulin (12 S, 134,000 daltons) (Gill and Tung, 1976) comprises the major rapeseed protein fraction (35%). Finlayson et al., (1969) reported that approximately 10% of the total seed nitrogen exists as a strongly basic globulin with a molecular weight of 13,500 daltons and a sedimentation coefficient of 1.7 S. Hydrophobic residues dominate the amino acid profile of rapeseed proteins, while basic and acidic amino acids are in relatively low concentration (Sosulski and Sarwar, 1973).

The role of oilseed protein products as food ingredients can be nutritional, functional or in many cases, both. Although oilseed proteins are not nutritionally "complete", they can serve as excellent sources of many essential amino acids (Sosulski and Sarwar, 1973). In many cases, however, it is functional and not nutritional qualities that determine the acceptability of the oilseed protein product to the consumer. As defined by Kinsella (1979), functional properties are "the intrinsic physicochemical characteristics which affect the behavior of protein in food systems during processing, manufacturing, storage and preparation".

Functional properties are controlled by the composition and structure of the proteins and the interactions of proteins with one another and with other substances. The characteristics can be modified or improved by deliberately altering the proteins. Wall (1979) found that denaturation of protein was beneficial in improving protein hydration, resulting in products with more desirable texture. Dalek et al. (1970), through work on vital wheat gluten, showed that drying methods strongly affected solubility. Spray drying was

found to cause mechanical damage to the secondary and quaternary protein structure, resulting in increased solubility. Decreased solubility due to thermal denaturation was reported by these authors for drum dried samples.

The remaining sections of this literature review deal with research reported by other authors in the field of protein modification for improved functionality. In addition, several functional properties will be discussed individually, outlining their theoretical bases and describing methodologies employed in their evaluation.

B. Enzyme and Surfactant Treatments

Enzyme hydrolysis of soy protein was investigated by Puski (1975) using a neutral protease preparation from Aspergillus oryzae. Protein hydrolyzed by this enzyme was found to have higher solubility, lower viscosity in aqueous dispersion and decreased gel-forming abilities. Pepsin hydrolyzates of soy proteins are used as foaming agents in confections and bakery products (Wolf and Cowan, 1971). The solubility of these hydrolyzates in the isoelectric range (pH 4 to 5) is much higher than unmodified proteins. Thus, in acidic foods such as citrus juices, these modified proteins could be incorporated without protein precipitation and sedimentation. Generally, the foaming power of the protein is increased by enzymatic treatment, however the foam stability is poor (Horiuchi et al., 1978). Nash and Wolf (1967) reported that small amounts of unhydrolyzed protein, added to the hydrolyzates, produce stable foams. Soy protein hydrolyzates have bitter and beany flavors which can be removed by ultrafiltration (Roozen and Pilnik, 1973). The use of enzyme hydrolysis to alter the properties of fish protein concentrate (Cheftel et al., 1971), cottonseed protein (Arzu et al., 1972) and egg albumen (Grunden et al., 1974) are also reported in the literature.

Catsimpoolas and Meyer (1971b) prepared dispersions of soy isolate containing various types and concentrations of lipid. They found that the viscosity of the dispersions increased with the concentration of triglyceride added. This effect was attributed to the arrangement of protein molecules around the lipid to form micelles. Polar lipids in flour have been used for many years to improve the mixing characteristics and strength of doughs. Wall (1979) cited enhanced aggregation and cohesion of the wheat gluten protein as responsible for the improvement.

Kobrehel and Bushuk (1977) found salts of fatty acids to be effective in solublizing freeze-dried glutenin. Canella et al. (1979) reported that surfactants disrupt hydrophobic bonds between proteins. Nakai et al. (1980b) attributed the solublizing action of anionic surfactants on oilseed proteins to the interaction between hydrophobic groups on the protein and the surfactant. These interactions would have the effect of increasing negative charges on the protein due to the anionic tail (carboxyl group), resulting in increased protein-protein repulsion.

C. Protein Solubility

Most globulins are insoluble in the range of pH near the isoelectric point, but are soluble in water or dilute salt solutions above or below the isoelectric point. It is generally assumed that association of protein molecules decreases solubility and dissociation increases solubility (Hermansson, 1973a). Ionizable amino acids play an important role in determining the electrostatic interactions between protein molecules, and therefore pH, salts and temperature influence the balance between attractive and repulsive forces that controls solubility (Wall, 1979).

The solubilities of complex protein systems differ considerably from the solubility behavior of native proteins. Processing techniques strongly affect solubility (Hutton and Campbell, 1977). After isoelectric precipitation, the proteins in a soy isolate are no longer completely soluble in a pH 7.6, 0.5 M phosphate buffer (Nash and Wolf, 1967). Thermal treatment during processing disorders the protein, permitting unfolding and the possibility of new interactions which may cause insolubility through aggregation. Hydrogen and hydrophobic interactions are reported by Hermansson (1973a) to be the major bonds affecting solubility.

The solubility behavior of a protein-rich product is often used as an index of the potential or limitations of the material as a food component (Kinsella, 1976). A high solubility is required, for example, in proteins which are to be added to beverages (Wall, 1979). Protein solubility is often used as an index of denaturation. Nash et al. (1971) observed a rapid decrease in solubility when soy proteins were denatured. However, Hermansson (1979a), using differential scanning calorimetry techniques to follow denaturation, observed that high solubility could be obtained from completely denatured soy proteins.

The techniques used to determine protein solubility are empirical, not easily duplicated, and often have little relevance to the behavior of the protein in a food system. The most commonly used procedure is called the Nitrogen Solubility Index (AOCS, 1969). Betschart (1974) defined the Nitrogen Solubility Index as "the proportion of nitrogen of a protein concentrate which is determined as soluble after a specifically defined procedure". The basic procedure has been described by Betschart (1974), Hermansson (1973a, 1973b) and Lawhon and Cater (1971). The protein is dispersed in water and the pH is

adjusted to some desired level. Centrifugation of the dispersion is followed by a determination of the nitrogen content of the supernatant. The determination is affected by the source, processing history, conditions of the solubility determination and the presence of other components (Shen, 1976). Hermansson (1973a) reported that solubility decreased as centrifugal forces increased. Centrifugal forces ranging from 1400 x g (Paulsen et al., 1960) to 40,000 x g (van Megan, 1974) have been employed. Increased protein concentration was found by Betschart (1974) to decrease solubility. Hermansson (1973a), however, found solubility differences of less than 1% between soy protein isolate concentrations of 1, 3 and 5%.

Modifications of the basic procedure have been suggested. Kinsella (1976) reported that measurement of solubility at several standard pH values might be used to obtain Nitrogen Solubility Profiles of proteins, which would be of greater value in predicting the solubility behavior in food systems. Lawhon and Cater (1971) recommended a heating step in the blending of the protein dispersion prior to centrifugation. Filtration of the supernatant rather than simply decanting is employed in the AOCS (1969) standard procedure and by other researchers (Betschart, 1974; Lawhon and Cater, 1971).

D. Water Hydration Properties

Bound water is defined by Kuntz and Kauzman (1974) as "that water in the vicinity of a macromolecule whose properties differ detectably from those of the 'bulk water' in the same system". Proteins are capable of binding large quantities of water due to their ability to form hydrogen bonds between water molecules and polar groups on the polypeptide chains. The degree of association with water depends to a great extent on the steric availability of

the polar groups. Therefore, denaturation of protein should increase the water binding abilities of proteins as compared to the native globular state. Chou and Morr (1979) reported that aggregation can reduce the ability of the protein to bind water by decreasing the availability of amino acids. However, they also stated that aggregation may increase water-protein interactions by forming a structural network capable of imbibing water. Water interacting with the protein in this manner can be described as "held" rather than "bound" water.

The process of hydration of a dry protein powder has been described by Chou and Morr (1979). Upon exposure to water vapor, water is adsorbed at polar sites, forming a monolayer of water molecules. This monolayer comprises the only truly "bound" water in the total system, and may range from 0.3 to 0.5 g H_2O/g protein (Fennema, 1977). Further association of water with the protein takes the form of multilayer adsorption, with the water molecules closest to the monolayer most strongly attracted. The proteins swell, and if they are soluble, swelling increases until there is sufficient water present to surround individual molecules and solubilize them. Excellent comprehensive reviews of the subject of protein hydration have been published by Fennema (1977) and Lumry (1973).

Methods for studying protein hydration reported in the literature are abundant, but diverse. Hermansson (1977) used moisture sorption isotherms to follow the swelling and solvation processes in a soy protein isolate and other protein powders. Hagenmaier (1972), using a similar technique, found a positive relationship between water binding abilities of several proteins and the number of hydrophilic amino acid groups minus the amide groups. He suggested that deamidation of oilseed proteins, which generally contain large amounts of amide nitrogen, might improve their water binding abilities.

Hansen (1978) found NMR to be a better method of following protein hydration than moisture sorption isotherms.

Fleming et al. (1974) measured the water absorption of sunflower and soy flours, concentrates and isolates by centrifuging 10% dispersions at 2500 RPM for 15 min. The water retained per gram of product was calculated after removal of the released volume of water. This method provides a measure of the amount of water "held" by the product. Balmaceda et al. (1974) recommended a similar technique involving lower product concentrations, higher centrifugation forces (18,000 x g) and a heating step in the mixing procedure.

After experiencing some difficulties with the more traditional methods of water hydration measurement, Quinn and Paton (1979) devised a simple, highly reproducible method that simulates more realistically actual food product applications in which water supply is limiting (eg. doughs). Using this new technique, they found a negative correlation between protein solubility and water hydration capacity values, which has also been reported by Hermansson and Akesson (1975) and Lin et al. (1974) using other techniques. Johnson (1970), however, found that this relationship held only for solubilities between 70% and 90%, and that below 70% solubility, water absorption decreased with decreasing solubility. Other factors which have been reported to affect water hydration of protein products are carbohydrate content and particle size distribution, which influences the rate of water absorption (Duerte, 1976; Johnson, 1970).

E. Rheological Properties

In dispersions, interactions of the dispersed phase with the solvent influence the flow behavior. A solution is more viscous than its solvent

alone because of the additional energy needed to overcome solute-solvent and solute-solute interactions (Frisch and Simha, 1956). The extent to which viscosity is increased by adding protein solutes to water, depends primarily on hydrodynamic properties (Lee and Rha, 1979).

Very low concentrations of rigid spheres suspended in a solution do not participate in any hydrodynamic interactions, and the viscosity of the suspension is described by

$$\eta_s = \eta_o (1 + 2.5\ q),$$

where η_s is the suspension viscosity, η_o is the liquid phase viscosity and q represents the volume ratio of the dispersed phase (Laztity, 1974). However, most proteins are not spherical and may be flexible, so that not only volume fraction but shape and flexibility also contribute to viscosity. Swelling of proteins increases the flexibility, effective hydrodynamic volume and axial ratio of the macromolecules, resulting in increased viscosity (Hermansson, 1972). Heating of proteins in dispersion will produce a similar viscosity-increasing effect due to dissociation (Kinsella, 1979).

During processing of oilseed protein products, conditions favoring denaturation may be encountered, often resulting in agglomeration of protein molecules to form aggregates. When placed in water, the size of the dispersed phase units can range from uni-molecular to large agglomerates. The particle size distribution affects the hydrodynamic volume and viscosity of the dispersion. Lee and Rha (1979) subjected soy protein dispersions to mild or vigorous blending regimes to produce dispersions with large or small sized aggregates, respectively. The dispersions with larger particles were found to be higher in viscosity than the small particle dispersions. These researchers also reported that hydrophobicity was extremely important in determining the size and shape of the dispersed protein.

Hermansson (1975) compared the flow behavior properties of dispersions of soy protein isolates processed under varying conditions. Isolates that had been produced under very mild extraction conditions exhibited flow behavior similar to that associated with dispersions of native protein, with low values of consistency coefficient (Power-law m value) and no yield values. Processes employing extremes of pH and/or temperature produced isolates which exhibited non-Newtonian flow in dispersion. She concluded that processing causes the formation of swelling aggregates that are responsible for the complex flow behavior. Lefebvre and Sherman (1977) measured the flow behavior of dispersions of sunflower protein products and found a negative semi-logarithmic relationship between Power-law coefficients m (consistency coefficient) and n (flow behavior index).

The viscosity of soy protein dispersions has been reported to increase exponentially with protein concentration (Circle et al., 1964). Deviations from this relationship at higher concentrations have been demonstrated by Lee and Rha (1979) who suggested that hydrophobic intermolecular interactions may be responsible. The effect of pH on viscosity has been studied by several researchers (Ehninger and Pratt, 1974; Ishino and Okamoto, 1975). Generally, as the pH of a soy protein dispersion is increased, the viscosity increases, up to pH 10-11. Above pH 11, alkali-denaturation occurs and the protein gels. Ehninger and Pratt (1974) found that the effect of pH was dependent upon protein concentration. Heating effects on the viscosities of 10% soy protein dispersions were found by Hermansson (1978) to be influenced by ionic strength.

Reversible time-dependence (thixotropy) was observed by Hermansson (1975) in 20% Promine-D soy dispersions. Lefebvre and Sherman (1977) found thixo-

tropic behavior in sunflower protein dispersions and reported that ageing of the dispersions resulted in a higher degree of thixotropy, due to the formation of stronger entanglements and linkages which required longer shearing times to disrupt.

Viscous behavior is exhibited as a result of the material dissipating applied mechanical energy, through flow. The ability to store energy reversibly during deformation is manifested as elasticity. Most fluid food materials exhibit behavior that is partially viscous and partially elastic and they are said to be viscoelastic materials (Mewis and Spaull, 1976). Even very dilute polymer solutions exhibit some degree of elasticity. Viscoelasticity is imparted by an interlinked network of dispersed molecules. In fluid foods, these interactions take the form of weak van der Waals forces while in solid materials, much stronger forces are involved. Rheological measurements of viscoelasticity must involve very small stresses and strains in order to minimize alteration of the internal network structure of the material during testing (Tung, 1978).

Fundamental studies of the viscoelastic properties of oilseed protein dispersions are relatively scarce. Isozaki et al. (1976) used creep compliance tests to measure the viscoelasticity of agar, egg albumen and soy isolate gels. In this method of testing, a small constant stress is applied to a sample and the resulting stain followed over time. The stress relaxation characteristics of 20% sunflower protein dispersions were measured by Lefebvre and Sherman (1977) using a Weissenberg Rheogoniometer. By imposing a predetermined constant strain on the sample and following the stress relaxation as a function of time, they concluded that the dispersions exhibited viscoelastic behavior.

Dynamic testing is another method of determining viscoelastic properties. Small, sinusoidally oscillating shearing deformations are applied to the sample and the viscoelastic moduli are determined from the amplitude ratio and phase shift between the stress and strain waves (Ferry, 1973). The viscoelastic properties of hydrated wheat gluten (Cumming and Tung, 1977) and rapeseed protein dispersions and gels (Gill and Tung, 1976, 1978) have been measured using dynamic testing. Although considerable attention has been focussed on the gelation of soybean proteins, much of the rheological work in this area has been done by measuring gel strengths and Brookfield consistencies, as opposed to the fundamental methods described above.

F. Gelation

Many food products have textural properties which are strongly dependent upon the ability of the components to form network structures (frankfurters, doughs, jellies). Although the gelation mechanisms of some food ingredients (polysaccharides and gelatin) have been comprehensively characterized (Mitchell, 1976), network formation by globular proteins is still not completely understood. Flory (1974) described a gel as having solid-like behavior, involving a certain degree of elasticity. For globular proteins, a gel can be identified as a state intermediate between a protein solution and a protein precipitate (Hermansson, 1978).

Globular proteins must be denatured before gelation can occur (Ferry, 1948). The denatured protein then aggregates, establishing protein-protein interactions. If the protein concentration exceeds some critical level, a balance between protein-protein and protein-solvent interactions is achieved, and a gel is formed (Hermansson, 1978). The rate of aggregation influences

the randomness of the intermolecular linkages. Highly ordered interactions produced by slow aggregation results in a gel with low opacity and high elasticity (Tombs, 1974).

Studies on gel-formation by oilseed proteins have been almost exclusively confined to soy proteins. Circle et al. (1964) used Promine-D to determine that gel stability depended primarily on protein concentration. Catsimpoolas and Meyer (1970) found that a minimum protein concentration of 8% was necessary in order to form a self-supporting gel network at pH 7 and room temperature. They also reported that the heating process disrupted the quaternary structure of the soybean globulins, activating the protein sol to the progel state (Catsimpoolas and Meyer, 1971a). Studies on the effects of pH and ionic strength on gel formation have been carried out by several researchers (Aoki, 1965; Catsimpoolas and Meyer, 1970; Hermansson, 1972; Ehninger and Pratt, 1974). The effects were found to be strongly dependent on the protein source and/or heating temperature.

Gelation of the 12 S rapeseed protein fraction has been studied by Gill and Tung (1978). Gels could be formed at the 4.5% protein level and the strongest gels were created under conditions of high pH and ionic strength. Gelling characteristics of fababean, field-pea and sunflower protein products were compared to the gel-forming abilities of soy products by Fleming et al. (1975). Isolate dispersions (10% protein) from all sources except sunflower formed gels of varying firmness at pH 7 after heating at 90^{o}C for 45 minutes and cooling in an ice bath. Heating dispersions (10% protein) of soy and sunflower protein flours resulted in increases in Brookfield consistency but did not produce gels.

G. Microstructure

Wolf and Baker (1975) used scanning electron microscopy (SEM) to study freeze-fractured soybean cotyledons and commercial soy flours, concentrates and isolates. They observed that some of the structural elements of the intact soy cotyledon (protein bodies and spherosomes) survived commercial processing. Gill and Tung (1976) examined rapeseed cotyledons and aqueous pastes of the 12 S protein fraction using both light microscopy (LM) and transmission electron microscopy (TEM). Evidence of protein agglomeration and a subunit structure were found by these researchers in samples of the 12 S protein fraction. Badley et al. (1975) studied the subunit structure of glycinin, the major storage protein in soybeans, using TEM.

Hermansson (1972) employed techniques in light microscopy to follow the swelling phenomena in Promine-D, caseinate and whey protein concentrate. Texture-structure relationships were investigated using SEM by Stanley et al. (1972) in rehydrated soy protein fibers and by Cumming et al. (1972) in extruded soy products.

Thermal aggregation and gelation of proteins have been studied using microscopy by several researchers. Tombs (1974), using TEM, found that the mesh structure produced by heating then cooling bovine serum albumin, was composed of protein strands arising from the aggregation of individual protein molecules. He compared gel pore size data calculated from theoretical considerations to measurements made from electron micrographs and found satisfactory agreement. TEM studies published by Saio et al. (1968) revealed the presence of numerous spheres, approximately 0.05 μm in diameter in an extract of soy proteins in the presence of $CaCl_2$. Upon heating, these spheres were observed to aggregate. Lee and Rha (1978) employed LM and SEM to

examine texture-structure relationships of soy proteins. They also observed destruction of the native protein structure on heating, and hypothesized that these structural changes were necessary to produce a gel network. Gels of the 12 S rapeseed protein fraction were examined using SEM by Gill and Tung (1978). These researchers reported that the gel pore size decreased, as the pH increased from 6 to 10. Hermansson (1979b) observed whey protein gels under the SEM and found that the addition of salt gave rise to a coarser structured gel. Powrie and Tung (1975) reviewed the use of electron microscopy in the study of immobilized water and discussed the microstructure of gels of milk proteins, gelatin and alginates.

MATERIALS AND METHODS

A. Source of Products

Samples of control and treated canola (rapeseed), soybean and sunflower protein products were available following completion of a two-year Agriculture Canada research contract awarded to Dr. S. Nakai and Dr. M.A. Tung of the Department of Food Science, U.B.C. As part of this contract research, canola meal (var. Tower), a commercially available soybean protein concentrate (Promosoy 100, Central Soya Co.) and sunflower protein concentrate supplied by Dr. F. Sosulski, Department of Crop Science, University of Saskatchewan were studied to determine the factors involved in protein insolubility. After investigating more than 30 different protein treatments for use during production of these high-protein products, three solubilizing agents were selected for pilot-scale treatment:

1. SDS (sodium dodecyl sulfate)
2. trypsin hydrolysis
3. potassium linoleate

The addition of 5% SDS was found to leave excessive levels of the agent bound to the protein in spite of exhaustive washing procedures. As the levels of residual SDS were higher than the level currently permitted by the Food and Drug Directorate, further studies on SDS-treated proteins were not conducted as part of this thesis.

Control, trypsin hydrolyzed and potassium linoleate treated protein products used in this study were prepared for the contract research studies according to the procedures outlined in Figures 1 and 2. Approximately 2 kg

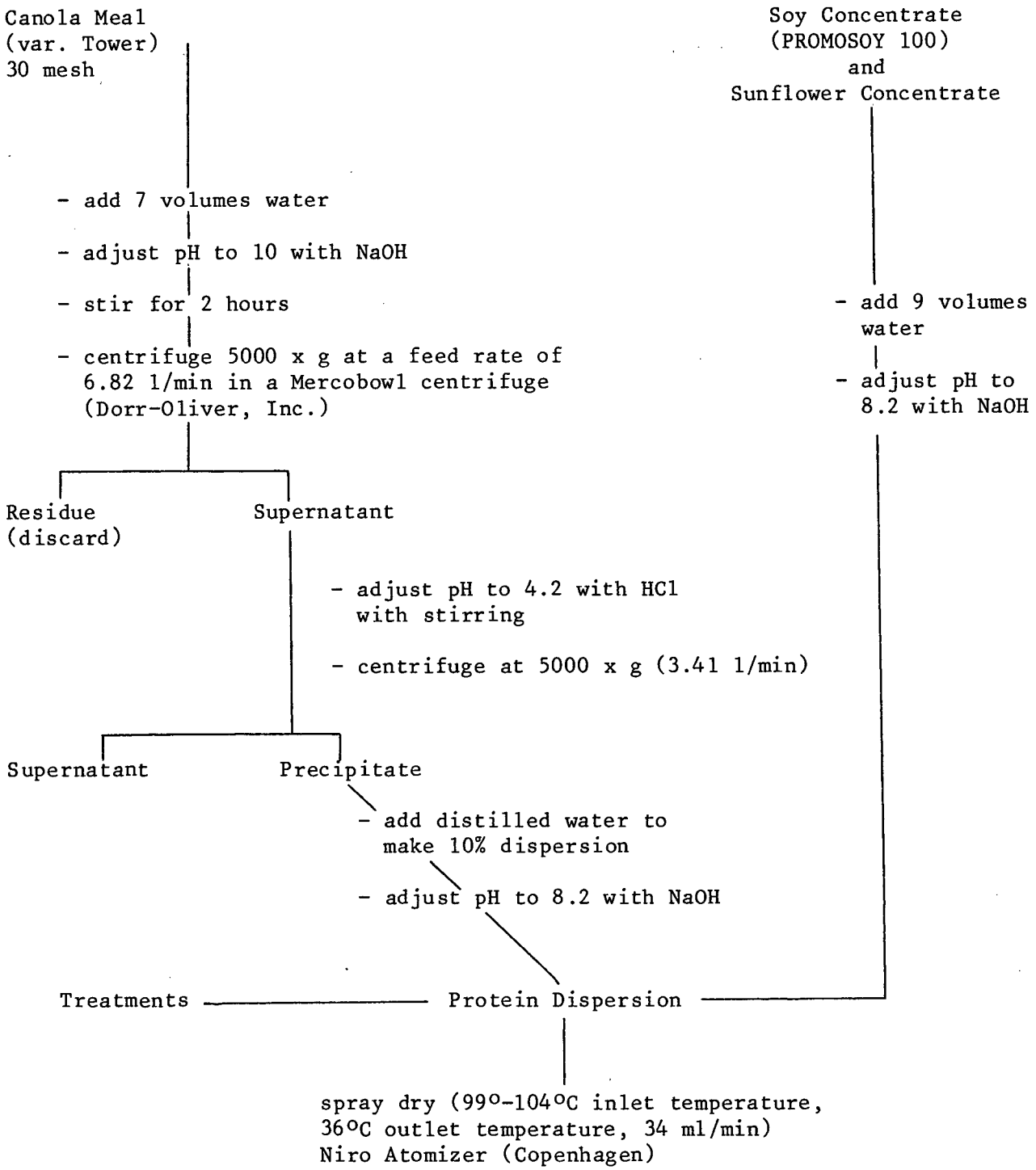

Figure 1. Preparation of protein concentrates and isolates.

Trypsin

- add trypsin (1% vs concentrate/isolate) (Fisher Scientific Co., Ltd.)
- heat at 60°C for 90 minutes
- heat at 90°C for 10 minutes
- quick cool
- readjust pH to 8.2 with NaOH
- spray dry next day

Linoleate

- add linoleic acid (8.7% vs concentrate/isolate) (Fisher Scientific Co., Ltd.)
- adjust pH to 8.0 with KOH
- adjust pH to 10.0 with NaOH
- heat at 65°C for 10 minutes
- readjust pH to 8.2
- spray dry next day

Figure 2. Procedure employed for treating the products with trypsin and linoleate.

of each of the nine different protein products were produced. After spray drying, the products were stored in opaque bottles at $4^{o}C$ until required for the various experiments performed in this study.

B. Chemical Composition

The nine products were analyzed for crude protein, total carbohydrate (as starch) and moisture content, to determine the effects of source and treatment on these parameters.

1. Crude protein

Protein analysis was carried out using a micro-Kjeldahl technique of Bradstreet (1965) and Concon and Soltess (1973). Using a Mettler analytical balance, 0.05 g samples of the protein products were weighed accurately into clean Kjeldahl flasks. A catalyst, 2.3 g of a K_2SO_4-HgO mixture (190:4, w/w), was added, followed by 2.3 ml H_2SO_4 (conc). The samples were digested by heating with periodic addition of H_2O_2 until all traces of organic material had been oxidized and the digest was refluxing halfway up the neck of the flask. When cooled slightly, the digest was diluted with distilled deionized water to 100 ml. An aliquot of this diluted solution was analyzed for % N using a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY). The % N values were converted to grams of N in the sample. Multiplication by 6.25 resulted in values for crude protein content which were expressed as percentages of the sample weights. Although it is known that the factor 6.25 overestimates the amount of protein in soy products (Wolf and Cowan, 1971; Balmaceda, 1974) this figure seems to be used for most oilseed protein analyses reported in the literature. The protein products

were analyzed in random order and each analysis was repeated twice to obtain three estimates of protein content for each sample.

2. Carbohydrate

The phenol-sulfuric acid method (Dubois et al., 1956; Pomeranz and Meloan, 1971) was used to estimate the percentage of total carbohydrates in the nine isolates and concentrates. Sulfuric acid was used to hydrolyze carbohydrate components in the sample to yield hexoses and pentoses, which reacted with phenol to produce a stable orange-yellow compound. The concentration of this compound was measured spectrophotometrically and related to total carbohydrate content by means of a standard curve. In this analysis, 50 mg ($\pm$ 0.1 mg) samples of the canola, soybean and sunflower products were diluted to 500 ml to yield suspensions containing approximately 20-240 µg/ml carbohydrate. Each suspension was prepared in duplicate, and 2 ml aliquots of each were transferred to 18 test tubes. To each test tube, 50 µl of 80% (w/v) phenol were added followed by the rapid addition of 5 ml H_2SO_4 (conc.). Immediately following this addition, the contents of the test tubes were agitated vigorously for 10 s using a vortex mixer. When cooled to room temperature, absorbance readings were taken at 485 nm using a Beckman Model DB Spectrophotometer (Beckman Instruments, Inc., Fullerton).

A standard curve was constructed by preparing suspensions of soluble starch containing 0, 20, 40, 60, 80, and 120 µg/ml carbohydrate, reacting 2 ml aliquots with 5 ml H_2SO_4 (conc.) and 50 µl of 80% phenol. Absorbance was measured at 485 nm.

3. Moisture

The AOAC vacuum oven method (AOAC, 1975, Section 14.002) was used to determine the moisture contents of the protein products. Samples (2 g) of each product were weighed accurately into nine pre-dried, desiccator cooled, weighed aluminum pans (70 mm diameter x 20 mm deep). The pans containing the samples were placed in a vacuum oven at 100°C and 84.4 kPa for 6 h. Cooling of the samples was accomplished in desiccators containing silica gel. After opening the desiccator following cooling, the samples were weighed as quickly as possible to prevent any appreciable gain of moisture on exposure to the room air. The samples were returned to the oven for an additional 12 h period, cooled and reweighed. Moisture contents were calculated from the dry weights recorded after this second drying period, although there did not appear to be any significant difference between the dry weights after 6 or 17 h of drying. The procedure was repeated, to obtain duplicate moisture determinations for each sample.

C. Structural and Functional Properties.

With the exception of water hydration capacity, all structural and functional properties of the protein concentrates and isolates were measured on 10% (w/w) dispersions of the products in distilled deionized water (7.5g product/75 g dispersion). The dispersions were prepared by hand mixing with a spatula for 1-2 min followed by blending/sonication for 1 h with a Polytron PT 10-35 blender (Brinkman Instruments, Inc., Westbury, NY) at a low speed (5) to minimize foaming. The blender was stopped two or three times during the mixing period in order to submerge and hydrate clumps of product floating on the surface of the dispersion or adhering to the blender shaft. The

dispersions were allowed to remain undisturbed for at least one hour after mixing to allow time for hydration of the dispersed solids. Functional testing of the dispersions was carried out on the day of preparation in order to minimize any ageing effects or bacterial degradation. Duplicate dispersions were prepared for each functional test performed.

A Fisher Accumet Model 420 Digital pH/Ion Meter (Fisher Scientific Company, Pittsburg, PA) was used to measure pH values of the dispersions. Conductance measurements on the samples were also taken to estimate the relative ionic strengths of the different dispersions. A YSI Model 31 Conductivity Bridge (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) was used for this purpose. A 25 cm long 2.5 cm diameter Pyrex cell containing a platinum-iridium electrode was immersed in the sample dispersions. A 117 V line voltage was used for the bridge supply. The reference cell resistance was adjusted to match that of the test solution. Conductance measurements were also recorded for several standard NaCl solutions, covering a range of ionic strengths from 0.01 to 1.

1. Structural properties

a) Light microscopy - dispersions

Sample dispersions were observed under a Leitz Labrolux microscope equipped with a Pentax ME 35mm camera. Unstained aliquots of the dispersions were contained on slides using cover slips sealed with petrolatum to prevent dehydration. Bright field, Kohler illumination was used and photographic images were recorded on Ilford FP4 black and white film. Dispersion samples stained with Lugol's iodine (Cook, 1974) were also examined to test for the presence of starch.

b) Scanning electron microscopy - thermal aggregation

Structural examination of the gel-like materials produced by heating the dispersions was accomplished using scanning electron microscopy techniques. Dispersion samples, approximately 5 ml, were heated in covered test tubes in a boiling water bath for 30 min and subsequently cooled in ice water. No significant moisture loss was noted by weighing the test tubes before and after the heating/cooling procedure. Ten ml of 5% glutaraldehyde fixative in 0.05 M phosphate buffer (pH 7.6) was carefully added to the cooled contents of each test tube. At room temperature the primary fixation was carried out for 20 h, at which time the fixative appeared to have penetrated half the depth of the semi-solid dispersion material. This procedure was adopted to achieve some degree of fixation of the material before any mechanical action was initiated to remove the dispersions from the test tubes. The partially-fixed material was cut into pieces, approximately 0.5 cm in length, and immersed into fresh 5% glutaraldehyde fixative overnight at room temperature. Two 15 min rinses in phosphate buffer were followed by secondary fixation in 1% osmium tetroxide in phosphate buffer for 90 minutes. Rinses of phosphate buffer (2 x 15 min) preceeded alcohol dehydration through a series of increasing ethanol concentrations (30 min each in 30, 50, 70, 95 and 2 x 100%). Replacement of ethanol with amyl acetate was accomplished through 15 min changes of 25, 50, and 75% amyl acetate in absolute ethanol, followed by 1 h in 100% amyl acetate. The samples of dispersion material were then dried in a Parr 5770 Critical Point Drying Bomb (Parr Instrument Co., Moline, IL) using carbon dioxide as the transitional fluid (critical temperature and pressure: 30^{o}C 7468 kPa). The dried fragments were fractured to expose an inner surface and mounted with silver paste and paint on aluminum stubs. A gold-

palladium alloy coating was applied to the samples with a Hummer Ion Sputtering Device (Technics, Inc., Alexandria, VA). An Etec Autoscan Scanning Electron Microscope (Perkin Elmer Etec, Inc., Hawyard, CA) equipped with a Polaroid camera was used to observe the structural detail of the samples and photographic images were recorded on Polaroid P/N 55 film. An operating voltage of 20 kV was employed.

2. Functional properties

Functionality tests used in this study were selected and adapted from the numerous tests reported in the literature. Those functional properties which appeared to measure the interactions of the concentrates and isolates in aqueous systems were of most interest.

a) Protein solubility

The amount of protein in the 10% dispersions that was "soluble" as opposed to simply dispersed was measured by centrifuging 20 ml of the dispersions at 10,000 x g for 30 min (Sorval Superspeed RC2-B, Ian Sorval, Inc., Norwalk, CT) and analyzing a 2 ml aliquot of the resulting supernatant for protein content using the micro-Kjeldahl technique of Concon and Soltess (1973). Although this technique is referred to as protein solubility in the scientific literature on functionality, the procedure in reality does not separate soluble from insoluble proteins. Rather a separation of sol particles is achieved, defined by a critical particle size. In this case, the technique provides measurement of the amount of protein in the dispersions present in small particles as compared to protein present in large aggregates. A centrifugal force of 10,000 x g was arbitrarily selected as an intermediate value of those reported in the literature (4,000 x g to 40,000 x g) (Briskey,

1968). From Stokes law, it was determined that a 10,000 x g force would cause particles greater than approximately 0.05 μm to sediment during the 30 minute centrifugation. Protein solubility was expressed as a percentage,

$$\frac{\text{g protein in 1 ml}}{(1.0\ \text{ml-g protein}/1.36\ \text{g ml}^{-1})} \times \frac{\text{g water added}}{\text{g protein in dispersion}} \times 100\%$$

b) Water holding capacity

The centrifuged dispersions used for protein solubility determinations could be used to obtain an estimate of water holding capacity of the protein products. By weighing the empty centrifuge tube and the centrifuge tube containing protein dispersion both before and after centrifugation and removal of supernatant, the weight of hydrated pellet was calculated by difference and related back to the weight of product in each dispersion. The water holding capacity was expressed as g H_2O held/g product. Although, as earlier discussed, there are some fundamental problems with this functionality test, these values provided an interesting comparison of the recently described water hydration capacity method of Quinn and Paton (1979) with the more traditional technique of water holding capacity.

$$\underset{\text{(g water/g product)}}{\text{Water holding capacity}} = \frac{\text{hydrated pellet (g) - product (g)}}{\text{product (g)}}$$

c) Water hydration capacity (WHC)

The method of Quinn and Paton (1979) was used to estimate the uptake of water by the products using a different technique than that used for water holding capacity. Samples of isolate or concentrate were weighed (± .01 g) into weighed 50 ml polypropylene centrifuge tubes. Approximately 25 ml of distilled, deionized water was added in increments, followed by mixing with a spatula to form an homogeneous paste. The tubes were centrifuged in the SS-34 Rotor of a Sorval Superspeed RC2-B centrifuge for 10 min at 2,000 x g. After

centrifugation, the supernatant was carefully removed and the tubes were weighed to determine the weight of the hydrated pellet. The approximate water hydration capacity was then determined as

$$\underset{\text{(g water/g product)}}{\text{Approximate WHC}} = \frac{\text{hydrated pellet (g) - product (g)}}{\text{product (g)}}$$

A series of 4 centrifuge tubes was then prepared for each product containing a constant amount of product in each, and varying amounts of water to encompass a range of water hydration capacities around the approximate water hydration capacity calculated (Table I). The weights and volumes of product and water, respectively, were calculated to yield a total weight of approx. 15 g. The centrifuge tube contents were mixed vigorously with a glass stirring rod for two min and all tubes were centrifuged for 10 min at 2,000 x g. In the series of tubes, some contained supernatant and others did not. The water hydration capacity was defined as the mean of the water hydration capacities represented by two adjacent tubes, one containing supernatant and the next without. The products were tested in duplicate in random order.

Table I. Example of water hydration capacity determination. (Sunflower-Trypsin: approximate WHC = 2.9 g H_2O/g product)

Tube #	Sample weight (g)	Water added (ml)	WHC (gH_2O/g product)	Supernatant
1	3.50	11.5	3.29	Yes
2	3.50	11.0	3.14	No
3	3.50	10.5	3.00	No
4	3.50	10.0	2.86	No

Water hydration capacity = 3.2 g H_2O/g product

d) Steady shear flow behavior

Sample dispersions (10% w/w) were subjected to rheological evaluation in steady shear using both a Brabender Rheotron coaxial cylinder viscometer (C.W. Brabender Instruments Inc., South Hackensack, NJ) and a Weissenberg Rheogoniometer cone/plate rheometer (Sangamo Controls Ltd., Bognor Regis, England). Duplicate dispersions were evaluated. For flow measurements with the Rheotron, 58 g dispersion samples were weighed into cup #A1 (radius = 2.8 cm) which was loaded into the instrument around the #A1 torsion sensing spindle (radius = 2.7 cm, length = 8.0 cm). Temperature control was maintained at $22^{o}C$ with a thermostatically controlled water supply circulating in a water jacket around the sample cup. The cup was rotated through a programmed loop of linearly increasing and then decreasing RPM for 200 s, representing a shear rate range of approximately 0 to 600 s^{-1}. Some of the samples were of such low viscosity that shear stress readings could be recorded only at shear rates greater than 30 s^{-1}. Shear stress and shear rate readings were recorded as voltages on a Digitec multichannel recorder (United Systems Corp., Dayton, OH), recording continuously through the shear rate program. Approximately ten shear stress-shear rate voltage data points were taken from each recorded output at logarithmically spaced shear rate intervals to calculate flow parameters. Calibration of the instrument was accomplished by measuring the shear stress-shear rate relationship of distilled water at $22^{o}C$.

Shear stress (σ,Pa), shear rate ($\dot{\gamma}$,s^{-1}) and viscosity (η,Pa.s) values were calculated from calibration data using a computer program written for this purpose for execution using the UBC Amdahl 470 V/6 computer. Also by computer, least squares linear regression was used to examine the fit of a Power-law model to the transformed data.

$$\log \sigma = \log m + n \log \dot{\gamma}$$

$$\text{or } \log \eta = \log m + (n-1) \log \dot{\gamma}$$

where m is the consistency coefficient (Pa s^n) and n is the flow behavior index. The Krieger-Maron correction for non-Newtonian behavior was applied to recalculate each shear rate value after an estimate of the flow behavior index (n) was evaluated from the data, assuming n = 1. Values of r^2 were calculated as a measure of the goodness of fit. The transformed data were plotted to visually examine for curvilinearity. Plotting also provided for detection of time-dependent flow in the sample which would be evident as a hysteresis loop formed by different shear stress values at a given shear rate when the shear rate was increasing as compared to values recorded as the shear rate was decreasing.

The Weissenberg Rheogoniometer was fitted with a #7 torsion bar (94 Pa $cm^3/\mu m$) and 7.5 cm diameter platens. The top platen had a nominal cone angle of 1^o and a gap set value of 44 μm. The flat bottom platen used was of the reservoir type, to contain excess fluid forced out of the gap during loading of the samples. Calibration of the instrument was checked using oil viscosity standard S6 (Cannon Instrument Co., State College, PA).

The bottom platen was rotated in both forward and reverse directions at several different speeds and the torque sensed by the torsion head transducer was recorded on a strip chart recorder. Tests for yield stress values were performed by stopping the rotation instantaneously with the brake and monitoring the declining shear stress readings as the sample relaxed. Calculations of shear stress, shear rate and viscosity were carried out by computer. Power-law parameters m and n and r^2 values and plots of the transformed data were obtained by computer using techniques similar to those

previously described for analysis of the data collected using the Brabender Rheotron.

The dispersions prepared and tested on the Rheotron were also evaluated with the Rheogoniometer that same day. Thus, a pooling of the Rheotron and Rheogoniometer results for each dispersion was possible to obtain 18 sets of m and n values for the duplicate tests of nine product dispersions. This was carried out using a computer program and the UBC computer. Values of apparent viscosity at 100 s^{-1} were calculated from the Power-law parameters.

e) Dynamic shear flow behavior

The Weissenberg Rheogoniometer was used to measure the viscoelastic properties of duplicate dispersion samples. The samples were loaded between a bottom flat plate which was oscillated through a small sinusoidally varying strain (amplitude 500 μm) and a top 1^{o} cone, 7.5 cm diameter platen supported by a #7 torsion bar. The frequency of the input strain was varied to assess the dependence of the viscoelestic parameters on frequency. A Tronotec Model 703A digital phase meter (Tronotec, Inc., Franklin, NJ) was used to monitor the amplitudes of the input (strain) and the output (stress) voltage signals, and the phase differences between the two signals.

From these data, values of storage modulus (Pa), relating shear stress to shear strain and values of loss tangent, reflecting the relative proportions of elastic to viscous natures of the samples were calculated by computer.

The viscoelastic parameters were measured at 20, 45, 70 and $95^{o}C$ in an attempt to follow rheological changes in the dispersions due to thermally induced aggregation. Temperature control was achieved by using a water jacketed lower platen assembly connected to a circulating water bath. Evaporation of the sample from the gap was minimized by applying a thin layer of 10 mPa s silicon oil to the exposed edge of the sample.

D. Statistical Analysis

1. Factorial analysis of variance

Data collected for all of the measured variables were coded for "source" (3 levels) and "treatment" (3 levels) and analyzed using an analysis of variance (ANOVA) program package (*MFAV) available for use on the UBC computer. Duplicate values were available for all variables, with the exception of the protein determination which was carried out in triplicate. Log transformations were performed on consistency coefficient and apparent viscosity (100 s^{-1}) data to achieve better homogeneity of variance, resulting in more meaningful statistical analysis.

Data for variables for which the source treatment interaction term was non-significant ($p>0.05$) were subjected to further statistical analysis using Duncan's multiple range test, available with the MFAV program. Duncan's multiple range test (Walpole, 1974) is a commonly used method for performing multiple comparisons among means. These analyses determined which sources and/or treatments were significantly different from each other in terms of each particular variable.

2. Single factor analysis of variance

The same data analyzed in the factorial analysis of variance were also treated as single factor "products" (9 levels) and analyzed in a one-way analysis of variance, using the *MFAV program. As there are no interaction terms in a one-way ANOVA, Duncan's mutliple range tests could be used to perform multiple comparisons among means for all variables.

3. Simple correlations

A correlation matrix involving means of 12 variables was generated using the statistical computer program MIDAS (Fox and Guire, 1976). Nine data points were available for each correlation. Scatter plots of several significant correlations were obtained by computer for visual examination.

RESULTS AND DISCUSSION

A. Composition

Mean protein, carbohydrate and moisture contents of the nine oilseed products are presented with their standard errors in Table II.

1. Crude protein

Results of the crude protein determinations (Table II) revealed that the canola products were higher in protein content than the soy and sunflower samples. The term "isolate" can be used to describe the canola control product, as the dry basis (db) protein content is greater than 90%. Soy and sunflower control products, containing at least 70% db protein, can be referred to as protein concentrates. The value of 70.0% db protein reported for the soy-control product shows good agreement with data supplied in a technical brochure for this product, Promosoy 100. The manufacturers of this product reported a range of protein content from 70.1 to 70.8% db (Central Soya, 1979). The protein content of the sunflower concentrate has been reported by Sosulski (1978) to be 73.5% db. A mean value of 74.6% db protein, obtained in this study shows satisfactory agreement.

Trypsin hydrolysis of the products during processing did not bring about a significant change in the measured protein contents as compared to control products. An increase in non-protein nitrogen would be expected as a result of hydrolysis, however the total amount of nitrogen in the product would remain unchanged. Nakai et al. (1980a) reported an increase in non-protein nitrogen from 2% db in control canola products to 42% db in canola product that had been hydrolyzed by trypsin at a level of 0.5% at pH 8.0 and 65^{o}C for 30 min.

Table II. Mean values of protein, carbohydrate and moisture contents of canola, sunflower and soybean products.

Product		Crude protein (% db)	Total carbohydrate (% db)	Moisture (% db)
Canola	- Control	91.3[a 1] (1.07)[2]	7.1[a] (0.015)	5.7[ab] (0.0951)
	- Trypsin	91.0[a] (1.84)	7.1[a] (0.365)	5.4[a] (0.0645)
	- Linoleate	84.0[b] (1.28)	5.9[a] (0.004)	7.2[d] (0.0703)
Sunflower	- Control	74.6[c] (1.17)	23.7[bde] (0.380)	7.2[d] (0.110)
	- Trypsin	74.6[c] (0.841)	22.2[bd] (2.65)	6.4[c] (0.0955)
	- Linoleate	69.8[d] (1.24)	20.1[b] (0.590)	6.1[bc] (0.220)
Soybean	- Control	70.0[d] (1.71)	27.3[ce] (1.095)	6.5[c] (0.257)
	- Trypsin	69.7[d] (1.73)	28.0[c] (1.855)	6.5[c] (0.0588)
	- Linoleate	60.2[e] (0.841)	25.8[cde] (0.950)	5.7[a] (0.00504)
		n = 3	n = 2	n = 2

[1]Means in a column sharing the same superscript are not significantly different ($p>0.05$) as determined by Duncan's multiple range test

[2]Standard error of the mean

The addition of linoleic acid at a level of 8.7%, brought about significant decreases in protein content over controls for all three sources. This decrease could be attributed to displacement of some weight of protein by the fatty acid. Nakai et al. (1980a) measured the amount of linoleate bound to soy and sunflower proteins following bench-top treatment of the oilseed products. They found that only 75 to 95% of the added linoleate was bound to the protein when the surfactant was added at levels of 8 to 10%. In pilot-scale products, such as those used in this study, the binding of linoleate to the protein may be even less complete, due to less efficient mixing techniques.

2. Carbohydrate

The standard curve derived from absorbance data collected for starch solutions was found to be well described by the regression equation:

$$A = (.0809 \times \mu g\ CHO) + .0601 \qquad r = .943$$

The carbohydrate contents of the samples were calculated using this equation. The canola isolates contained much less total carbohydrate than the sunflower or soybean concentrates (Table II).

Glycoproteins present in the samples would contribute to the total carbohydrate measured in this analysis. The 12 S globulin of canola is a glycoprotein containing approximately 8% carbohydrate (Gill and Tung, 1976). Wolf (1970) reported that the 7 S protein fraction in soy contains 6% carbohydrate.

Treatment of the products with trypsin or linoleate did not significantly alter the carbohydrate contents as compared to controls. There was, however, a trend towards lower carbohydrate contents in linoleate-treated samples, a result which would be expected due to displacement of some carbohydrate by the linoleic acid.

3. Moisture

The range of moisture contents determined for all samples was found to be 5.4 to 7.2% db (Table II). Although there were significant differences among products, no trends were noted. During spray drying of the products, the target moisture content ranged from 3 to 5% wet basis as determined using a moisture balance. The observed differences in moisture content may not be related to treatment effects.

4. Other components

Components not accounted for by the above analyses would be lipid (including linoleate in the linoleate treated samples) and ash.

B. Structural Properties

1. Light microscopy - dispersions

Micrographs depicting the nine dispersions are presented in Figures 3 to 11. In the canola control dispersion (Figure 3), aggregates were observed which were approximately spherical in shape and which ranged in diameter from 1 to 75 μm. As over 90% of the dry solids in this product was found to be protein, these aggregates were assumed to be of proteinaceous material. The trypsin-treated canola dispersion (Figure 4) is observed to contain aggregates of smaller mean particle size (maximum diameter approx. 25 μm). The breakdown of large polypeptides to smaller molecules as a result of enzymatic hydrolysis may reduce protein-protein interactions responsible for aggregation. Treatment with linoleic acid brought about an increase in mean aggregate size (Figure 5). The long chain fatty acids may have initiated the formation of micelle-like structures, with the linoleate buried within a shell of protein.

The sunflower control dispersion (Figure 6) contained fragments of cell wall material which were irregularly shaped. These fragments ranged in size from less than 10 μm in length to over 100 μm. Smaller particles (<5 μm) in the dispersion were assumed to be aggregates of protein and simple polysaccharides. The presence of starch was indicated by the purple-blue color of some small particles following staining of dispersion samples with Lugol's iodine. Treatment of the sunflower concentrate with trypsin or linoleic acid did not alter the structural properties of the dispersions to any great extent (Figures 7 and 8). The cell wall fragments would not be expected to be affected by the treatments, and the protein aggregates were too small to permit observation of any changes in size.

Figures 3 to 5. Light micrographs of 10% aqueous dispersions (photo width (pw) = 500 µm)

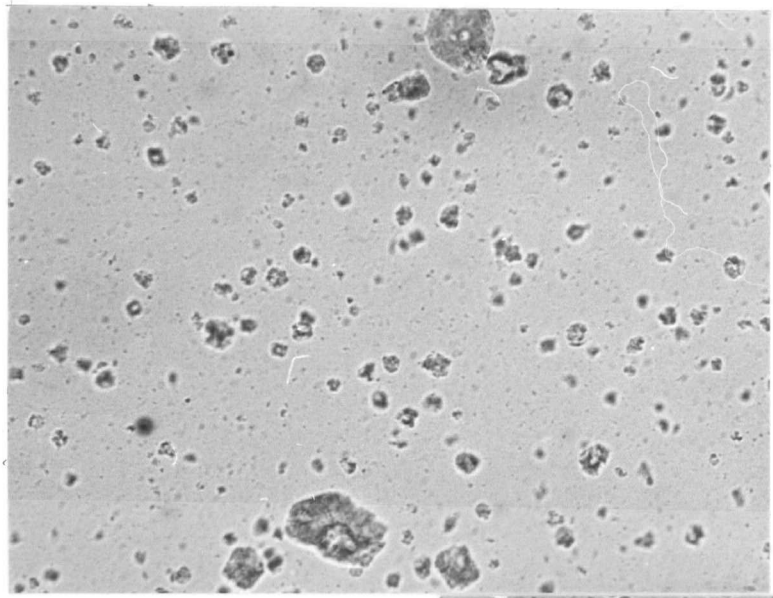

Figure 3
Canola control

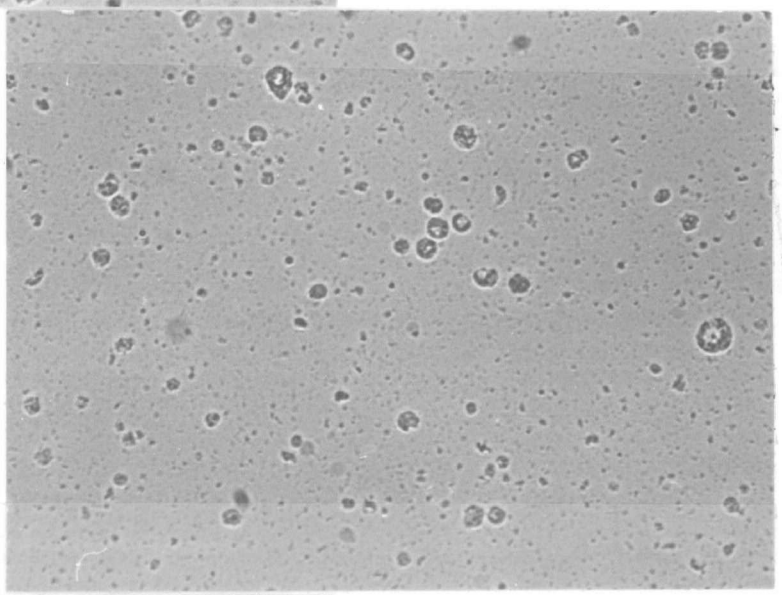

Figure 4
Canola trypsin

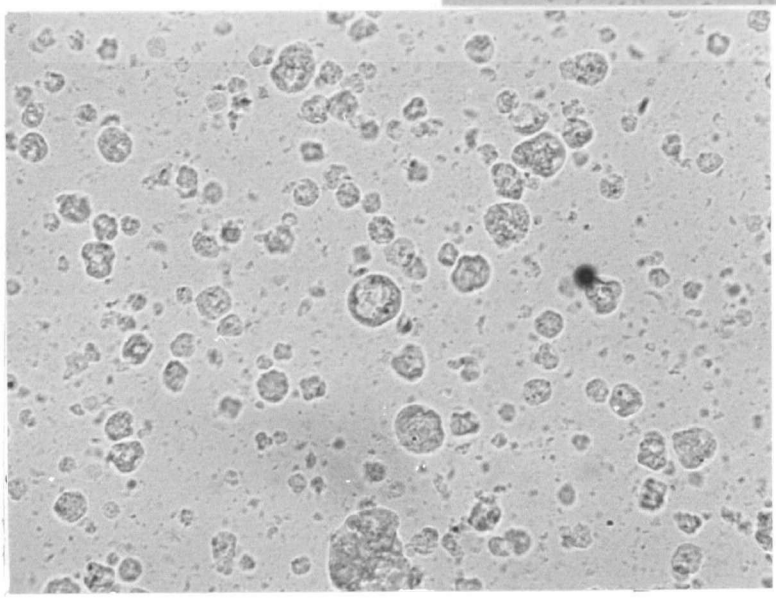

Figure 5
Canola linoleate

Figures 6 to 8. Light micrographs of 10% aqueous dispersions (pw = 500 μm)

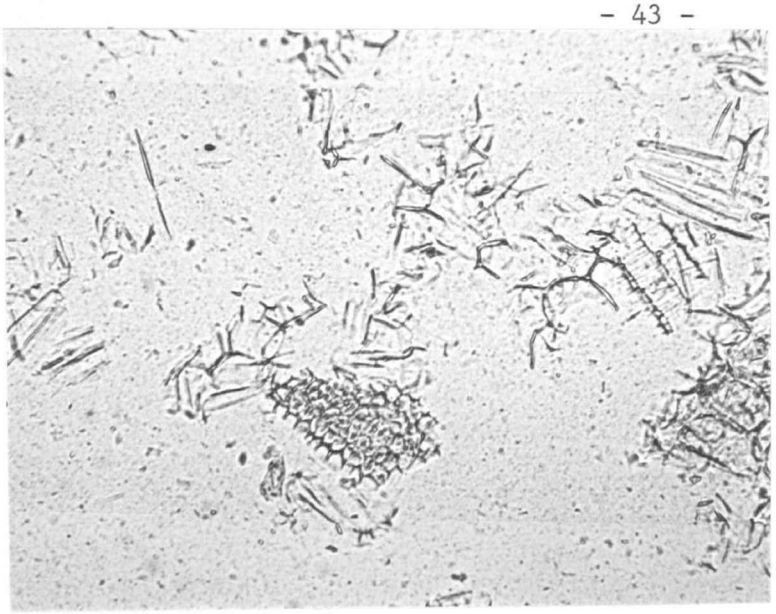

Figure 6
Sunflower control

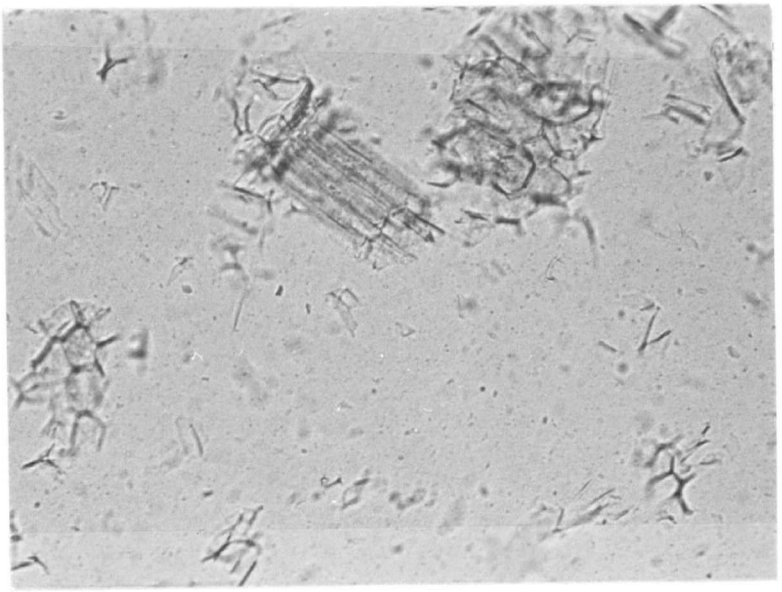

Figure 7
Sunflower trypsin

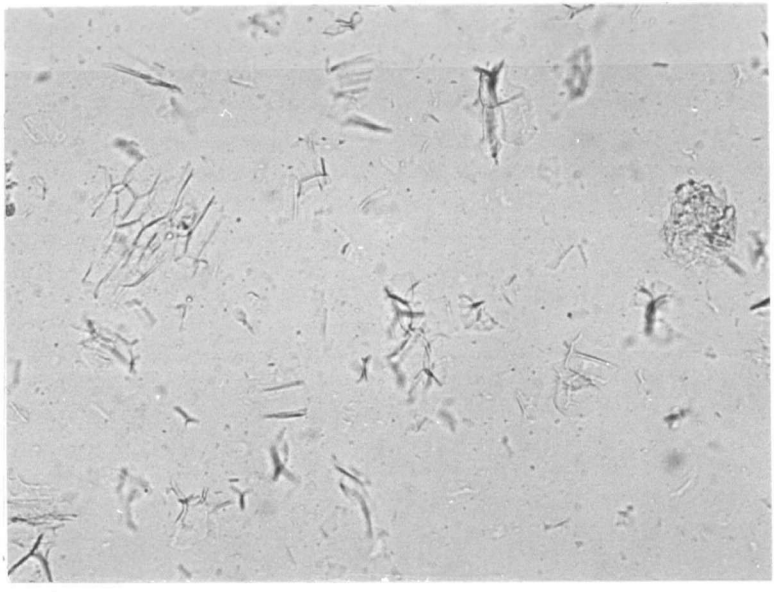

Figure 8
Sunflower linoleate

Figures 9 to 11. Light micrographs of 10% aqueous dispersions (pw = 500 μm)

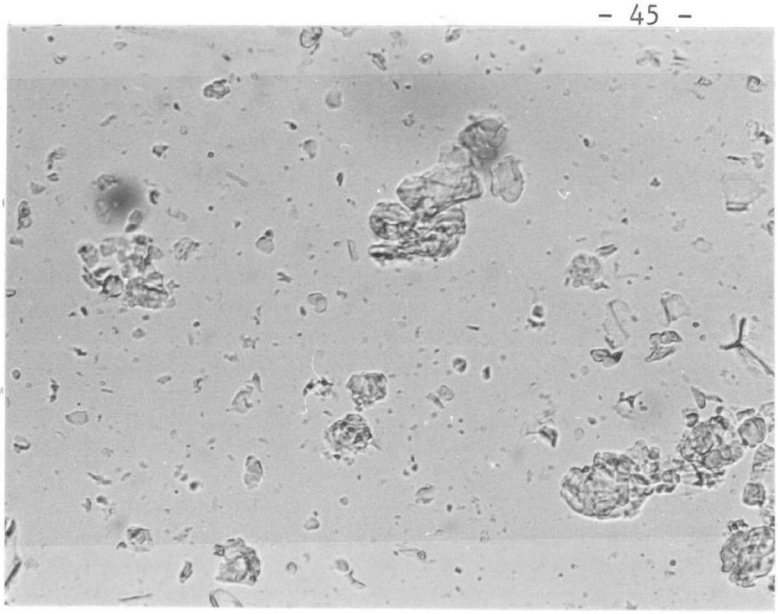

Figure 9
Soybean control

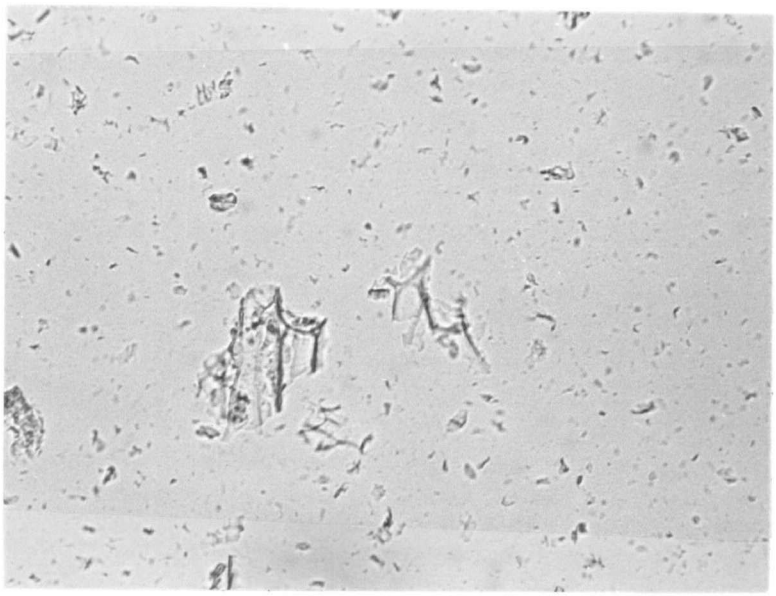

Figure 10
Soybean trypsin

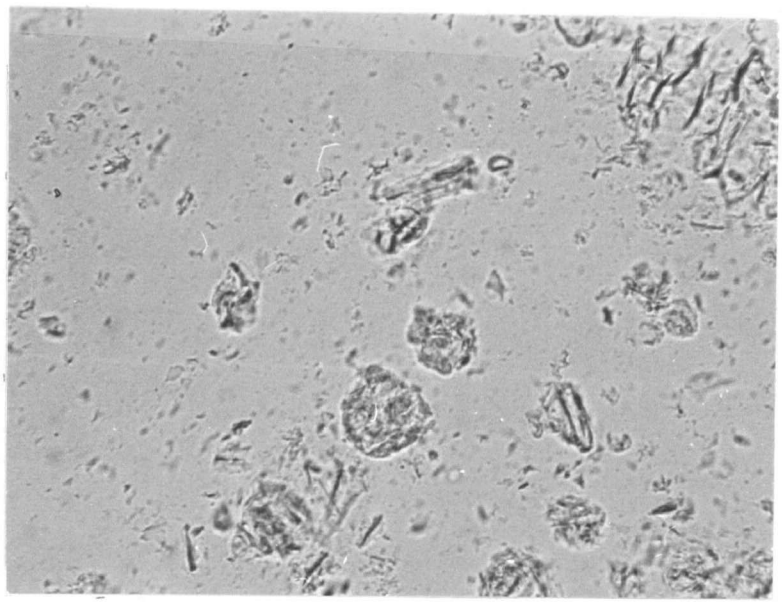

Figure 11
Soybean linoleate

The soy control dispersion (Figure 9) contained some fragments of cell wall material, however some of the larger particles (approx. 100 μm) did not have the characteristic cell structure and were assumed to be aggregates consisting predominantly of protein. Hydrolysis by trypsin reduced the size of the protein aggregates (Figure 10). The large particles appeared to be cell fragments. The linoleate-treated soy dispersion (Figure 11) contained large aggregates similar to those observed in the control dispersion. The interaction of starch with linoleate and protein may be contributing to the formation of these large particles. Pomeranz and Chung (1978) studied the interactions of lipid with soy protein and carbohydrate in breadmaking and found that polar lipids serve as an adhesive, establishing hydrogen bonds with the starch and hydrophobic or hydrogen bonds with the protein.

Efforts to measure the particle size distribution of the dispersed phase by a number of different methods (filtrafugation, Coulter counter, sieving and micromensuration) were unsuccessful. Clumping and dilution of the particles which occurred during these measurement procedures was believed to influence the size distributions, thus providing inaccurate estimates of the particle size distributions in the 10% dispersions.

2. Scanning electron microscopy - thermal aggregation

Scanning electron micrographs of the gel-like materials produced by heating the sample dispersions in a boiling water bath, are presented in Figures 12 to 25. Examination of the canola-control samples (Figures 12 and 13) revealed the presence of small protein aggregates (<1 μm diameter) interacting to form the larger aggregates observed in the aqueous dispersions under the light microscope. In trypsin-hydrolyzed canola samples (Figure 14),

Figures 12 and 13. Scanning electron micrographs of heated 10% aqueous dispersions.

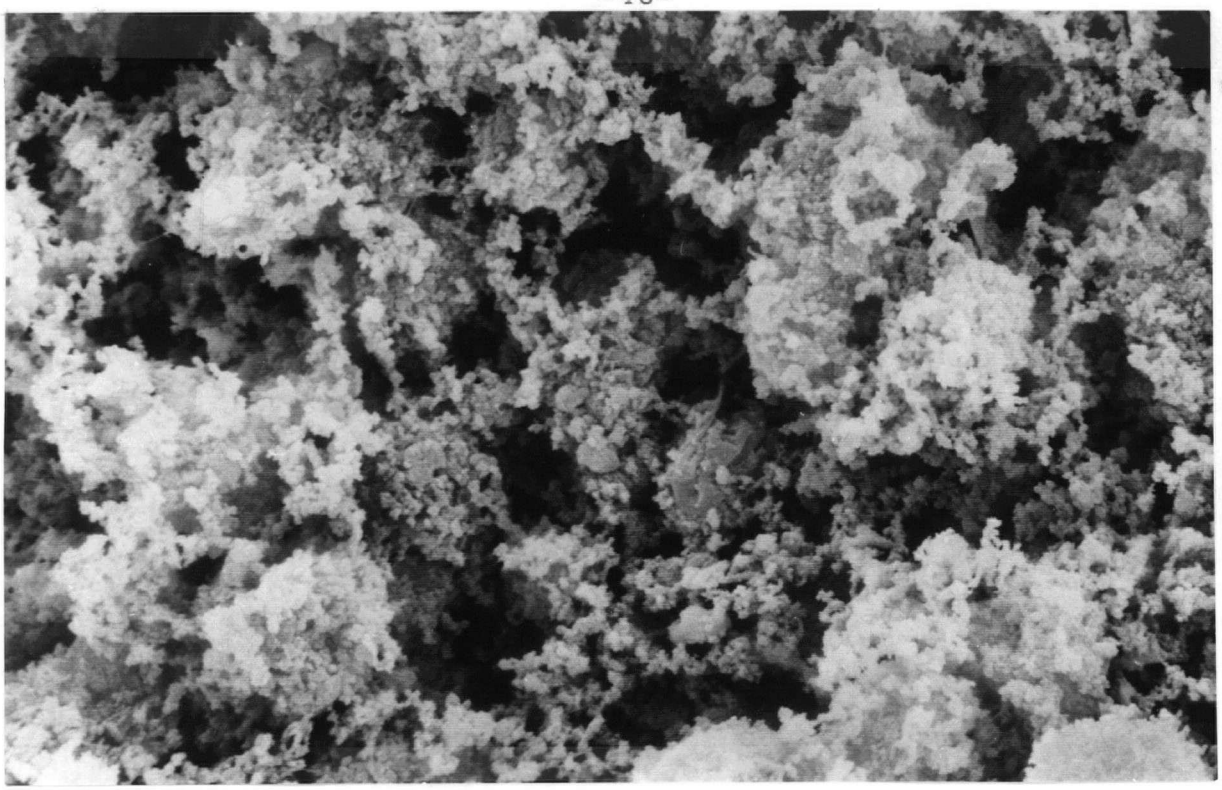

Figure 12. Canola control (pw = 60 μm).

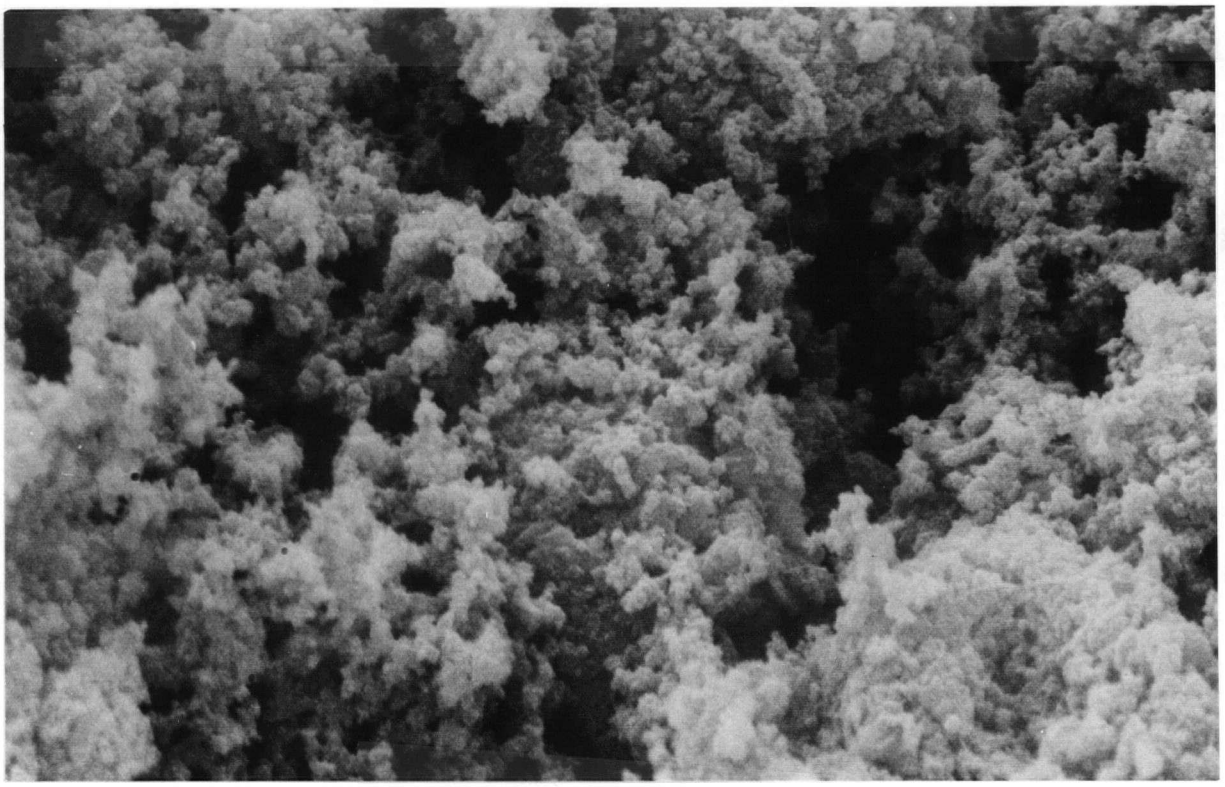

Figure 13. Canola control (pw = 20 μm).

Figures 14 and 15. Scanning electron micrographs of heated 10% aqueous dispersions.

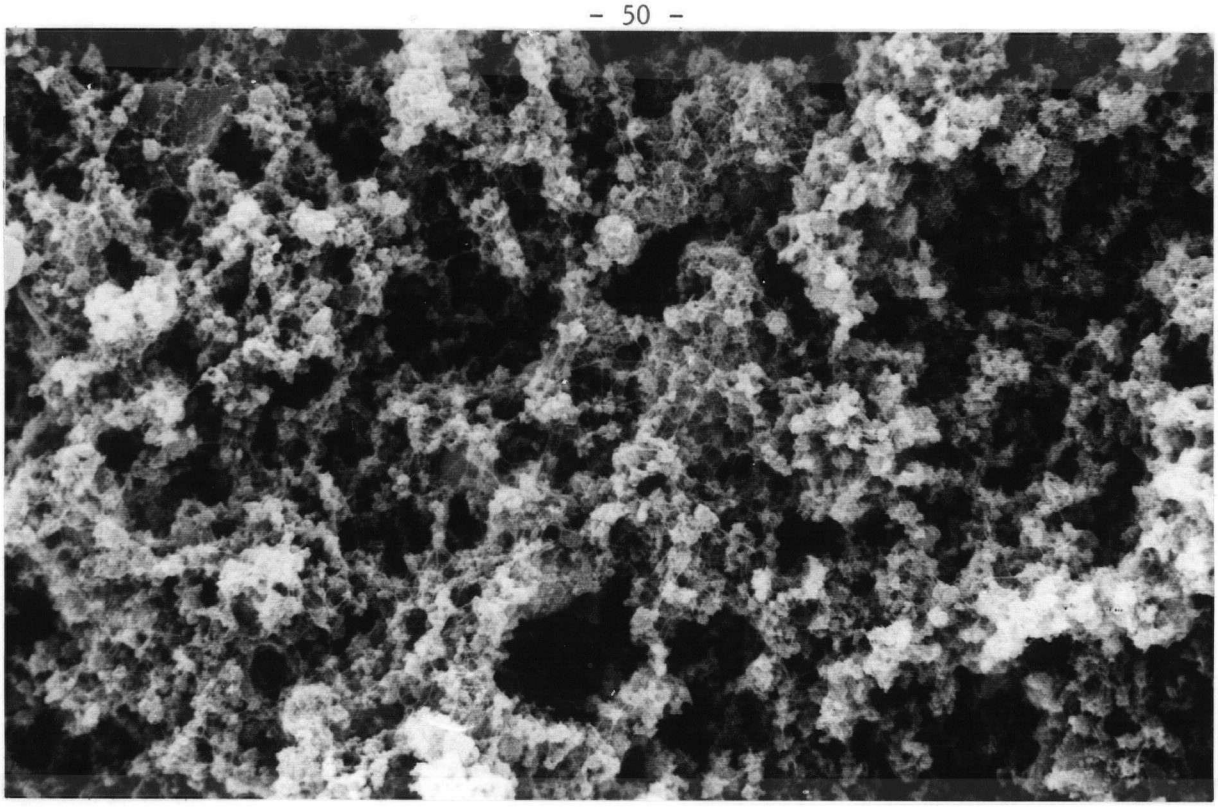

Figure 14. Canola trypsin (pw = 60 μm).

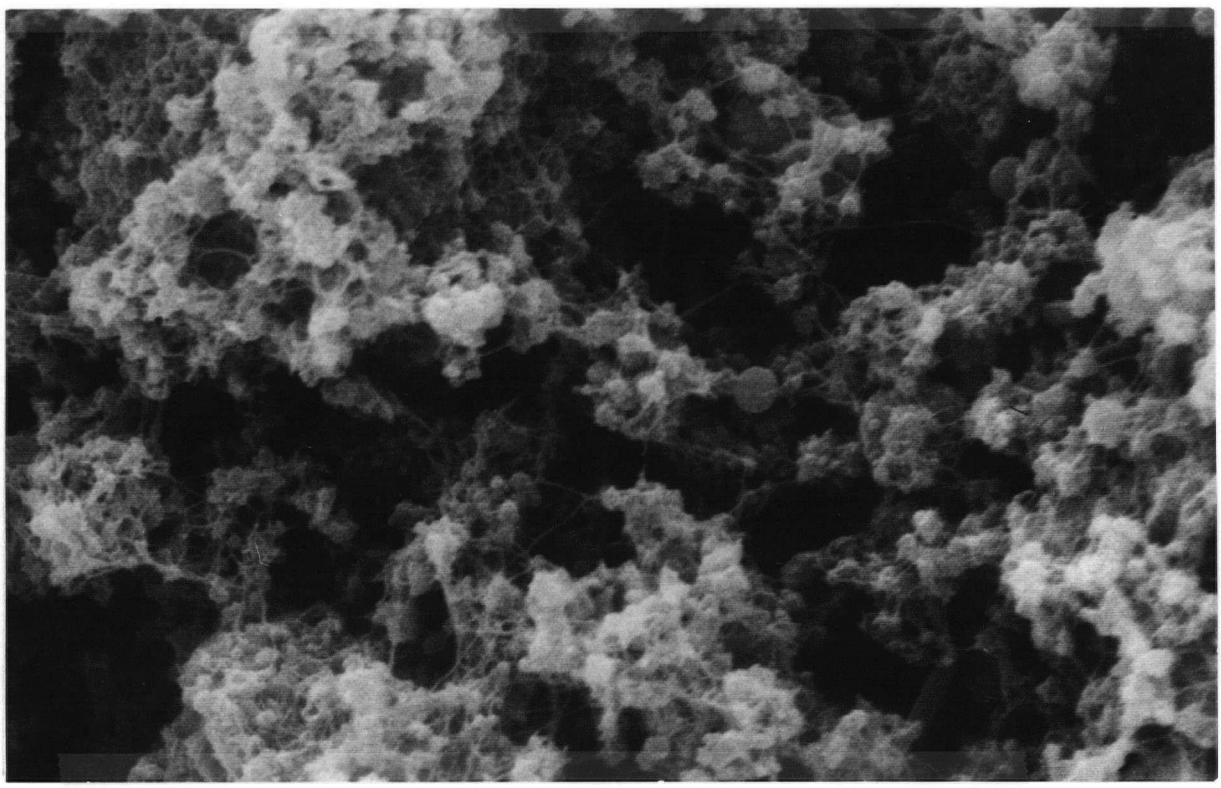

Figure 15. Canola trypsin (pw = 20 μm).

Figures 16 and 17. Scanning electron micrographs of heated 10% aqueous dispersions.

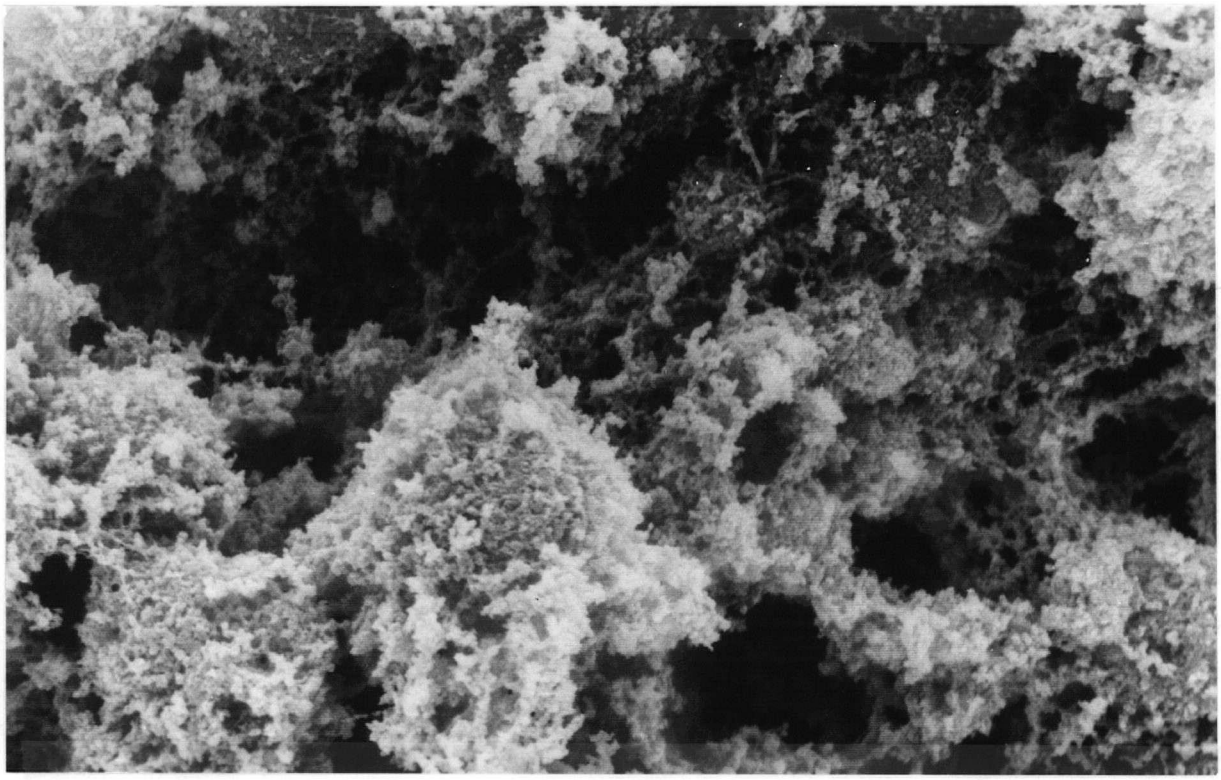

Figure 16. Canola linoleate (pw = 60 μm).

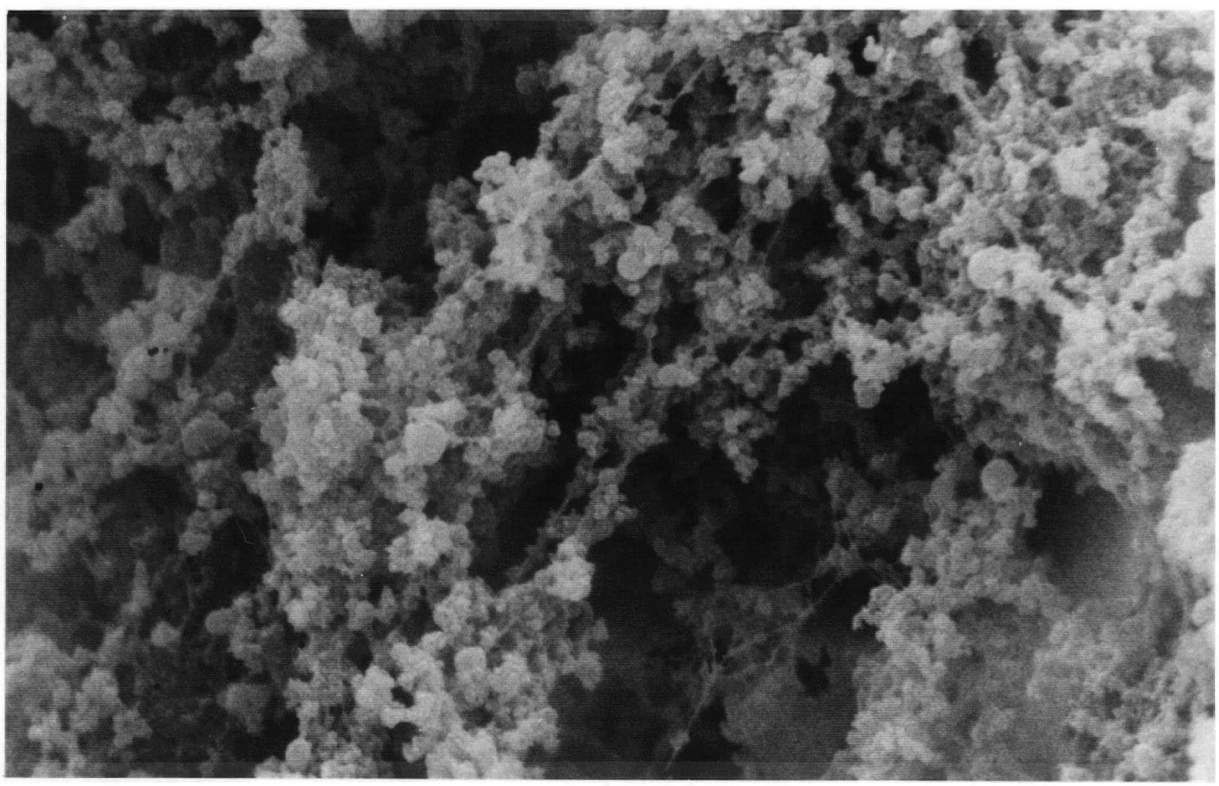

Figure 17. Canola linoleate (pw = 20 μm).

Figures 18 and 19. Scanning electron micrographs of heated 10% aqueous dispersions.

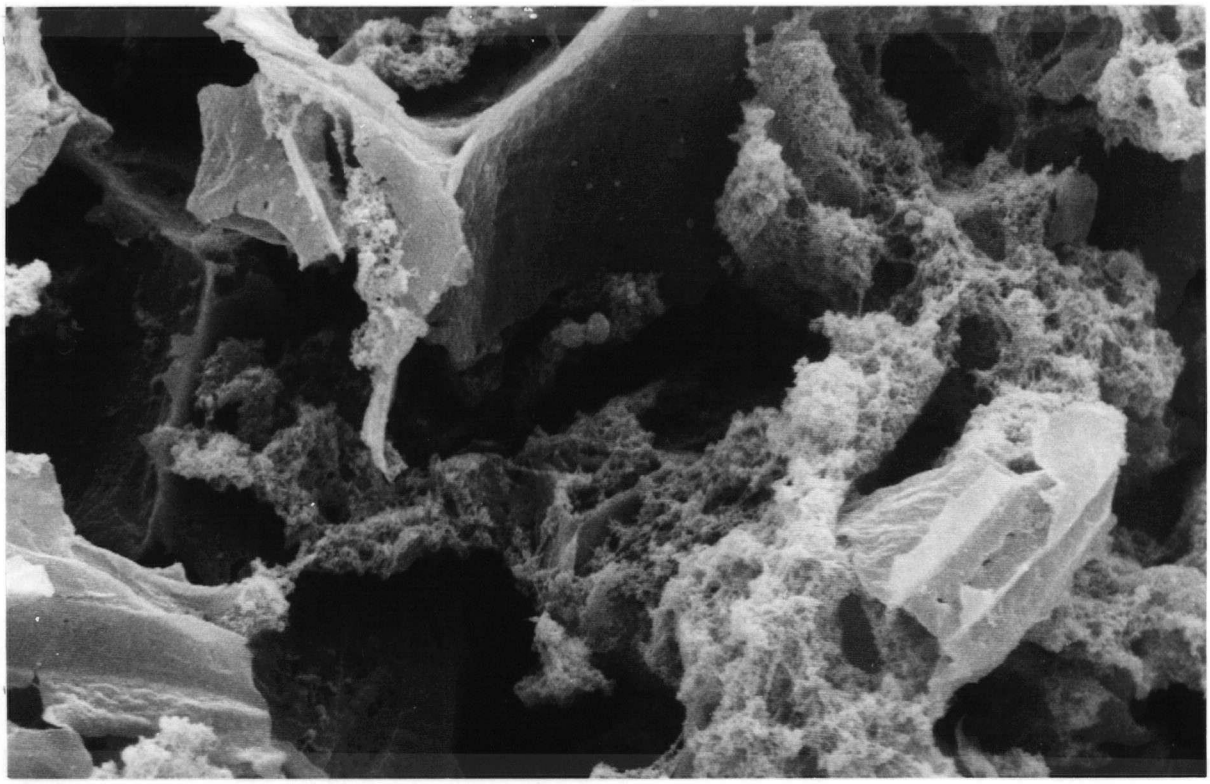

Figure 18. Sunflower control (pw = 60 µm).

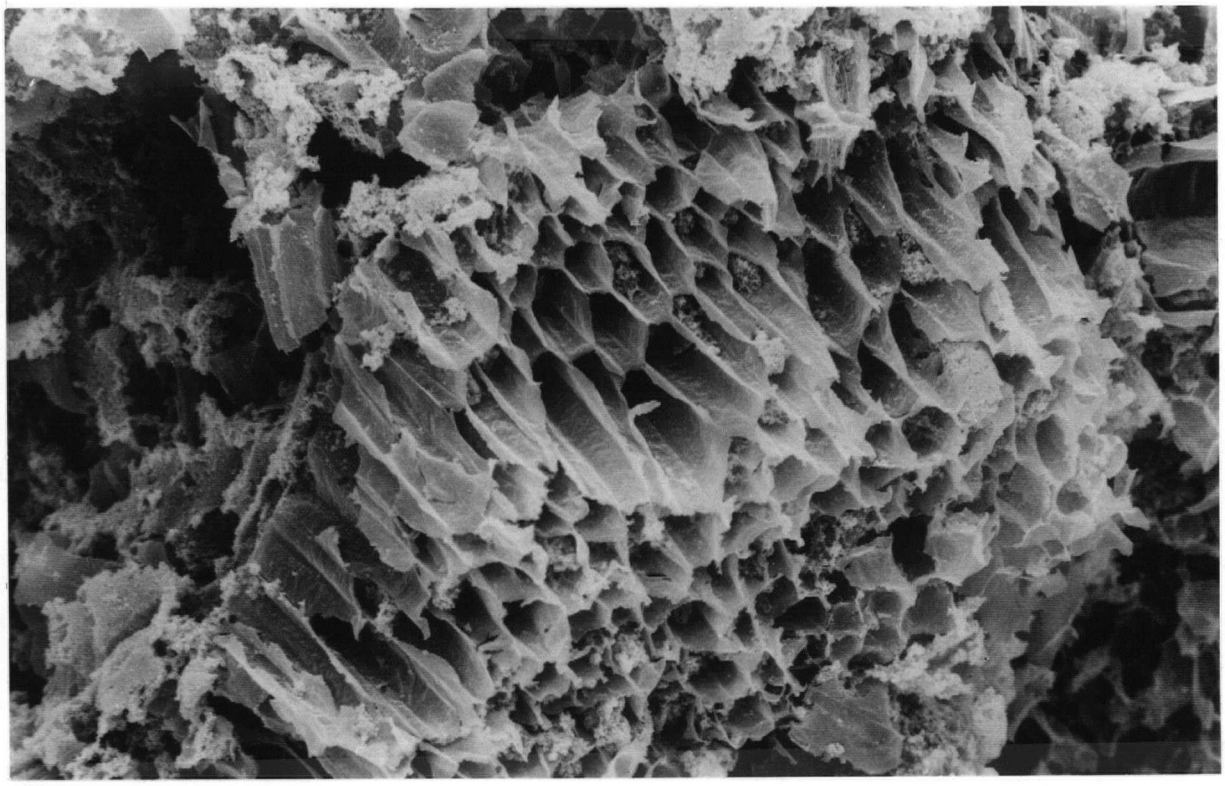

Figure 19. Sunflower control (pw = 300 µm).

Figures 20 and 21. Scanning electron micrographs of heated 10% aqueous dispersions.

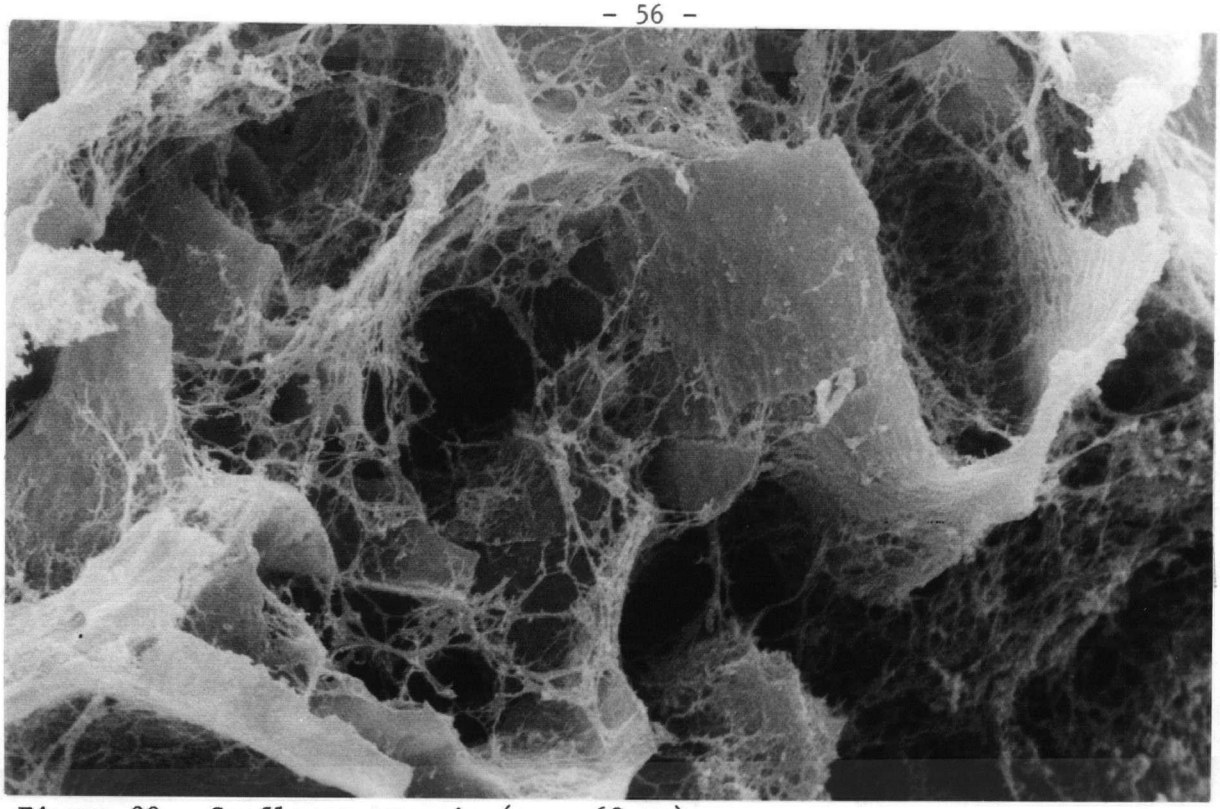

Figure 20. Sunflower trypsin (pw = 60 μm).

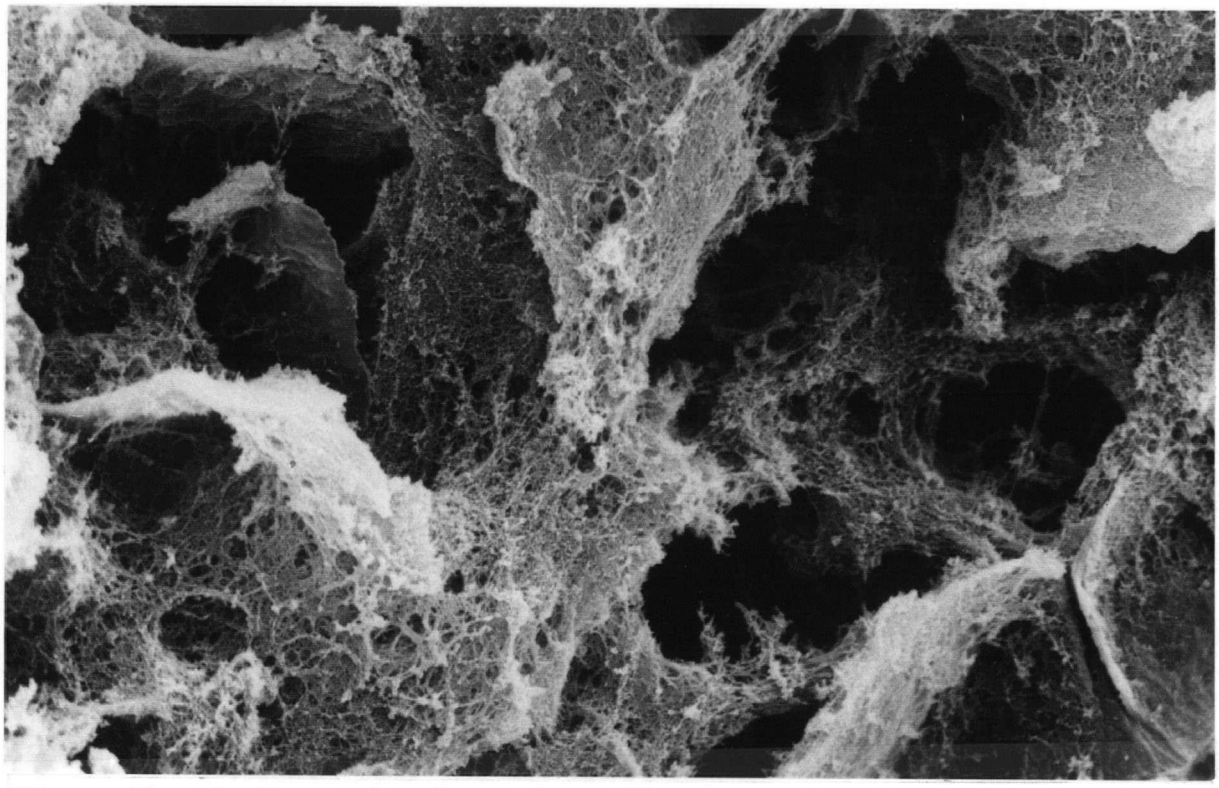

Figure 21. Sunflower linoleate (pw = 60 μm).

Figures 22 and 23. Scanning electron micrographs of heated 10% aqueous dispersions.

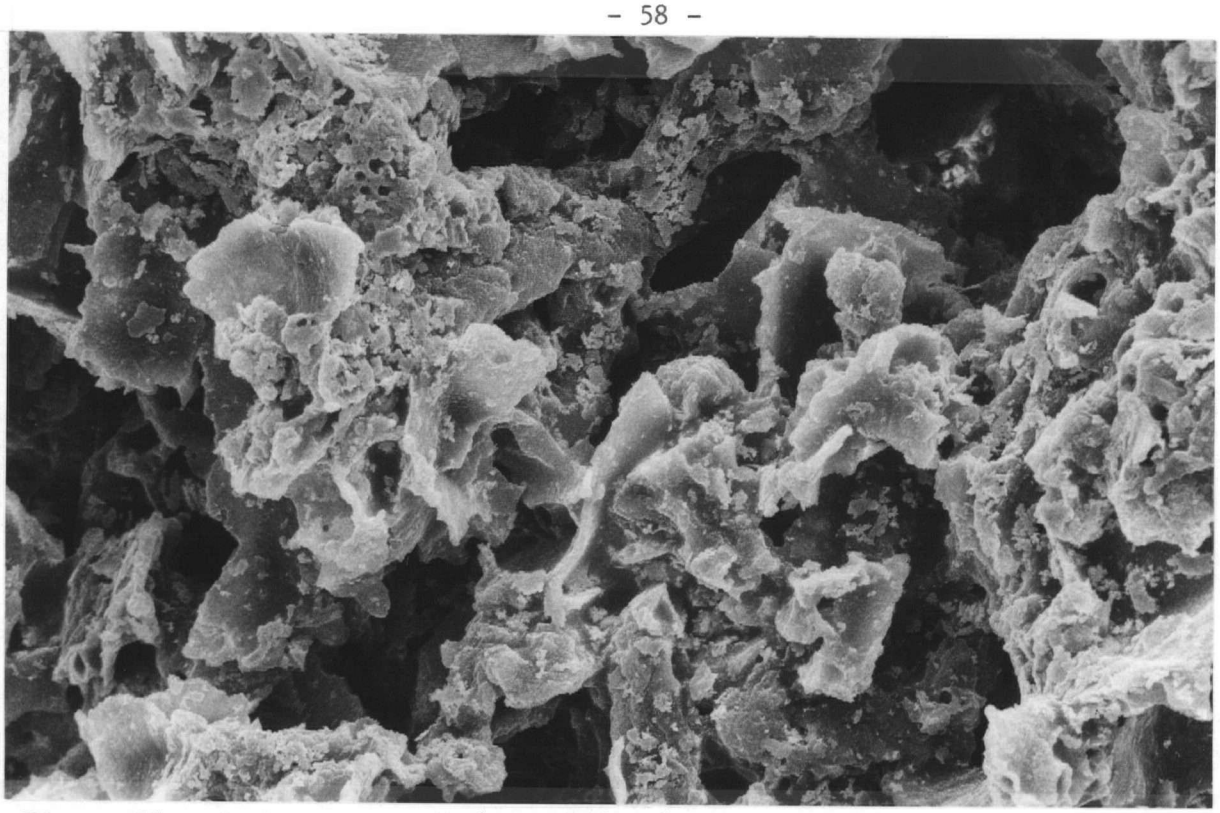

Figure 22. Soybean control (pw = 150 μm).

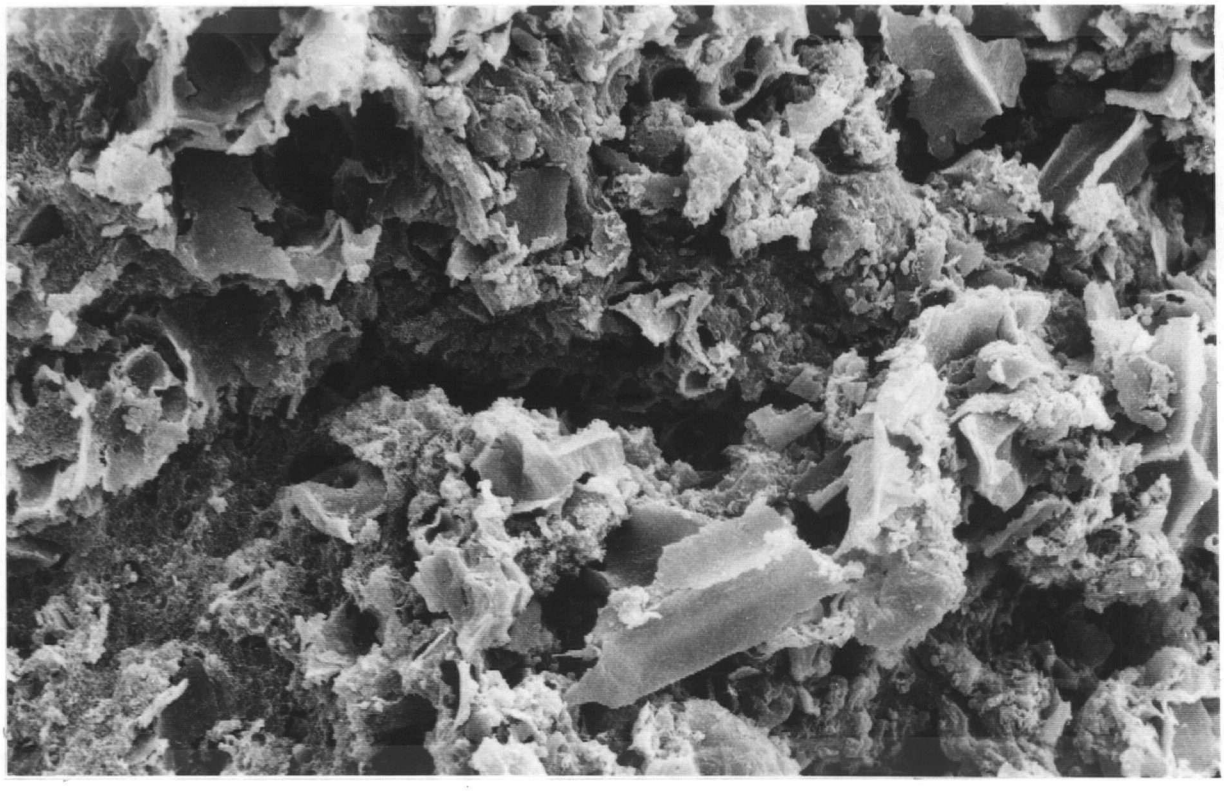

Figure 23. Soybean linoleate (pw = 300 μm).

Figures 24 and 25. Scanning electron micrographs of heated 10% aqueous dispersions.

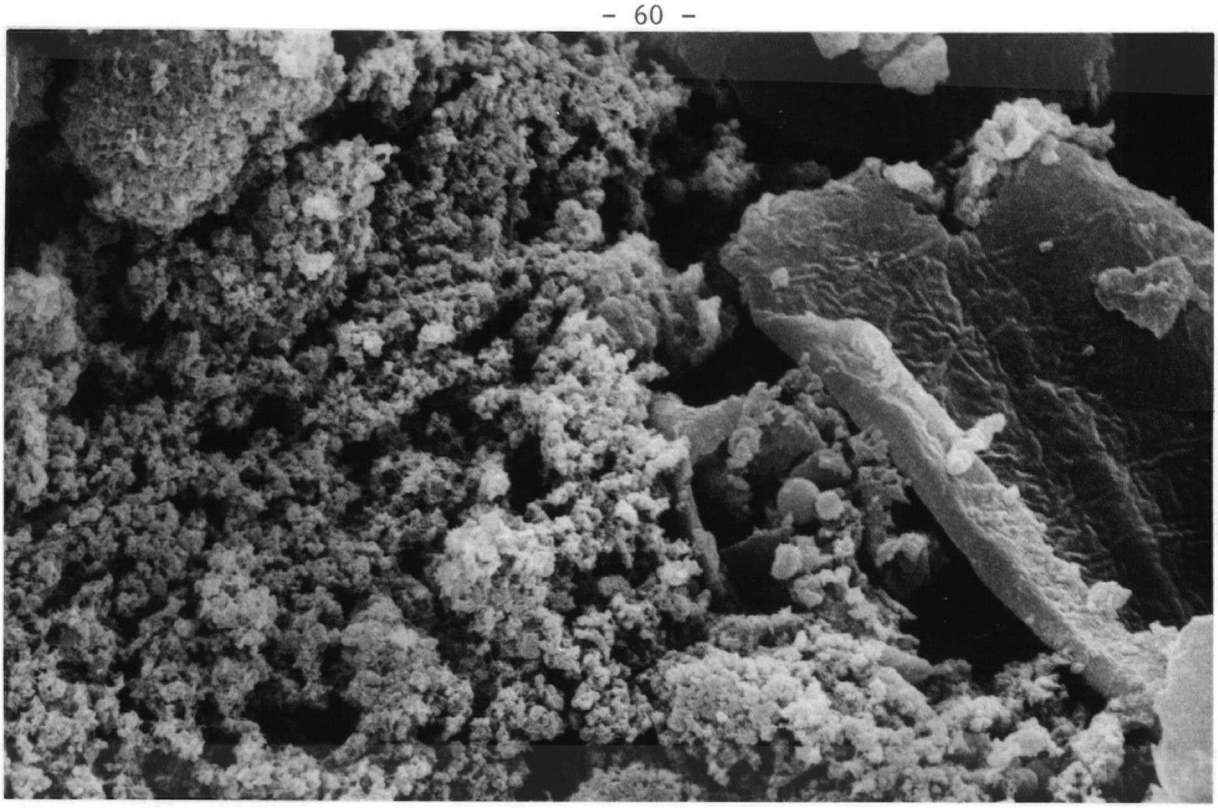

Figure 24. Soybean linoleate (pw = 60 μm).

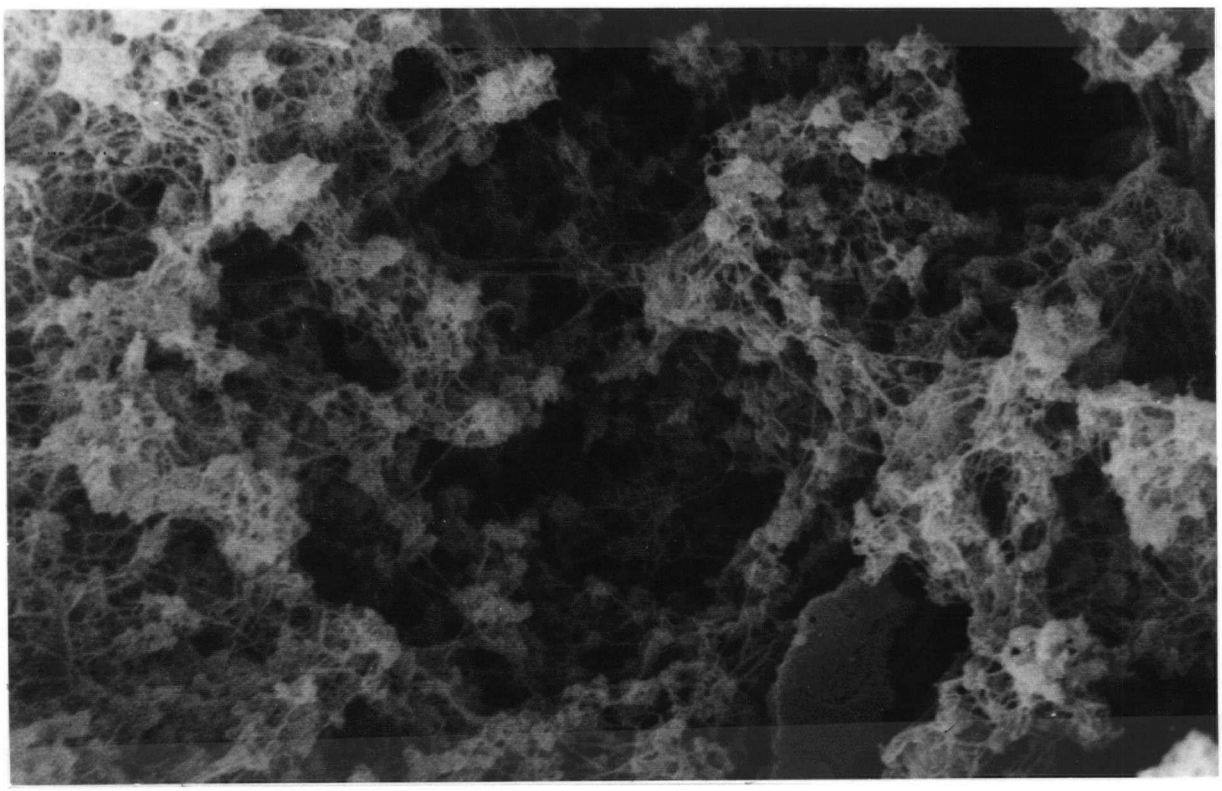

Figure 25. Soybean linoleate (pw = 20 μm).

a finer texture was observed as a result of a more uniform distribution of individual aggregates throughout the matrix. This structure would suggest more protein-protein interactions. Observation of the samples at higher magnification (Figure 15) indicated the presence of fine filamentous fibers linking protein aggregates, in the trypsin-treated canola material. It is possible that similar fibers were present in the canola-control samples, but due to the strong nature of the protein-protein interaction, were fewer in number and hidden from view . The linoleate-treated samples (Figure 16 and 17) contained linkages between protein aggregates that appeared stronger than those observed in the tryspin-hydrolyzed samples. A more elastic texture might be expected. The canola gel samples had similar structural properties to soy protein isolate gels examined by Lee and Rha (1978).

In samples of heated control sunflower material, a gel-like substance was found to be distributed throughout the cell wall fragments (Figure 18). This gel-like material may be composed of protein or carbohydrate, or both. However, the major non-protein constituents of oilseed concentrates are insoluble carbohydrates which are unlikely to form a network structure. Therefore the components involved in the formation of the matrix were assumed to be predominantly protein. Figure 19 shows a large piece of cell wall material, probably a fragment of the sunflower seed hull.

A finer-textured protein matrix was observed throughout the trypsin-hydrolyzed sunflower samples (Figure 20). Linoleate treatment produced sunflower gels with strong interactions (Figure 21) similar to those observed in linoleate-treated canola samples (Figure 16).

A gel-like matrix could not be located in the soybean-control samples examined (Figure 22). Some degree of gelation must have occurred in order to

produce the semi-solid textured material on heating, however, much of the protein in this product is insoluble and would not be expected to form the characteristic gel matrix observed in the canola and sunflower products. Possibly, polysaccharides are participating in the structure-forming process through gelatinization, exerting a stabilizing effect on a weak protein matrix.

The soybean-trypsin dispersion did not form a sufficiently solid structure on heating to withstand mechanical damage on transfer from gelling tube to fixative, and therefore examination of structural details was not possible for this sample. Examination of soybean-linoleate samples indicated the presence of a gel-like substance distributed throughout the cell wall fragments (Figure 23). At higher magnification (Figures 24 and 25) the matrix was observed to have a fine texture, similar to that observed in the canola-trypsin samples.

Visual examination of the samples after heating revealed that the linoleate-treated materials had more rubbery textures and shiny, translucent appearances as compared to corresponding control and trypsin-treated samples. These observations lend support to the results obtained from SEM examination which suggest that stronger protein-protein interactions were initiated by linoleate treatment. The participation of the long chain fatty acids in establishing and maintaining linkages between protein aggregates may be at least partially responsible. Tryspin hydrolysis might be expected to increase the opportunity for the proteins to interact, by exposing more amino acid side chains. However, results of both SEM and visual examination indicated that the interaction was fairly weak, and sensitive to mechanical disruption.

C. Functional Properties

1. pH

Measurements of dispersion pH were taken to determine whether differences observed in other functional properties could be attributed to a pH effect. During processing of the products, the pH was adjusted to 8.2 just before spray drying. Due to the strong buffering capacity of the protein, a stable pH value was difficult to attain. When the spray dried products were re-dispersed in this study, and pH measured, values ranging from 7.3 to 8.4 were recorded. Means and their standard errors are reported in Table III. Although there were significant differences among some of the means reported, the differences could be attributed to deviations in pH adjustment during processing, rather than to treatment effects.

2. Conductance

Conductance values for the 10% dispersions were measured as an index of ionic strength, to determine if the observed effects of treatment on other functional properties could be attributed to the effect of ionic strength. The results of the analyses are presented in Table III. Comparing the control dispersions, canola and soy samples have similar conductance values, while sunflower ranks lower in conductance. Conductance measurements were also recorded for several NaCl solutions of different ionic strength (0.01-1.00). The values recorded for the control samples, correspond to ionic strengths of approximately 0.04M NaCl (canola and soy) and 0.01M NaCl (sunflower).

Treatment of the products with trypsin or linoleic acid produced dispersions with significantly higher values of conductance than corresponding

Table III. Mean values of pH and conductance for 10% aqueous dispersions of canola, sunflower and soybean products (n = 2).

Product		pH	Conductance (μmho)
Canola	- Control	8.4[a 1] (0.0251)[2]	3370[a] (7.01)
	- Trypsin	7.6[b] (0.212)	4180[b] (12.0)
	- Linoleate	8.2[a] (0.00499)	4745[c] (3.49)
Sunflower	- Control	7.6[b] (0.0150)	1030[d] (6.89)
	- Trypsin	7.9[bc] (0.0395)	2145[e] (9.49)
	- Linoleate	8.1[ac] (0.00997)	2755[f] (7.50)
Soybean	- Control	7.7[b] (0.0200)	3450[a] (18.0)
	- Trypsin	7.5[b] (0.0349)	3870[b] (7.01)
	- Linoleate	7.6[b] (0.0399)	5590[g] (9.01)

[1]Means in a column sharing the same superscript are not significantly different ($p>0.05$) as determined by Duncan's multiple range test.

[2]Standard error of the mean

control dispersions. In the case of trypsin hydrolysis, this would be caused by the increase in hydrogen ion concentration as peptide bonds were cleaved. Linoleate treatment involved adjustment of pH to 10.0. In both treatments, therefore, more acid or base would be required to readjust the pH to 8.2, resulting in higher ionic strength than controls.

3. Protein solubility

Protein solubility determinations on the 10% aqueous dispersions were performed to assess the amount of protein in the dispersions that was present in small colloidal particles (<0.05 μm) as opposed to large protein aggregates. Solubility values (Table IV) were calculated on the basis that all of the water added to the dispersion would be available for solublizing protein. This assumption will lead to an overestimate of solubility, as some of the added water is chemically bound to insoluble protein and carbohydrate and is unavailable for solublization.

The three control products had significantly differing solubilities. The low solubility of the soy control product might suggest the presence of strong attractive forces, resulting in considerable aggregation of protein and carbohydrate components. Aggregation would be expected to decrease solubility through sedimentation and reduced availability of solvation sites.

Trypsin hydrolysis was found to be effective in increasing the solublity of canola, sunflower and soybean proteins. This proteolytic enzyme cleaves peptide bonds adjacent to basic amino acids. This action could dissociate protein structure within the aggregates in such a way as to increase interfacial area, exposing more charged and polar sites on the proteins to the surrounding water.

Table IV. Proportion of protein in 10% aqueous dispersion present in soluble state as particles <0.05 μm in size (n = 2).

Product		Mean (g protein soluble/100 g total protein)	Standard Error
Canola	- Control	56.6[a] [1]	.514
	- Trypsin	70.4[b]	.308
	- Linoleate	55.2[a]	1.35
Sunflower	- Control	79.3[b]	5.31
	- Trypsin	90.1[c]	.462
	- Linoleate	100.9[d]	5.38
Soybean	- Control	18.3[e]	1.11
	- Trypsin	58.7[a]	1.76
	- Linoleate	91.1[c]	4.29

[1]Means sharing the same superscript are not significantly different ($p>0.05$) as determined by Duncan's multiple range test.

The effect of linoleate treatment on solubility was found to vary with source. Sunflower and soy products treated with linoleic acid exhibited significant increases in solubility over controls. Salts of fatty acids have been reported to disrupt protein-protein hydrophobic bonds (Canella et al., 1979; Wall, 1979), which may affect solubility by increasing interfacial area. Nakai et al. (1980b) attributed the solubilizing effects of surfactants to the surrounding of proteins by negatively charged fatty acid carboxyl groups. The solubility of the canola product, however, remained unchanged following linoleate treatment.

4. Water hydration and water holding capacities

The water hydration capacity (WHC) method of Quinn and Paton (1979) was found to be an effective method for assessing the abilities of the products to hold water. Errors associated with the conventional water holding technique due to the discard of protein in the decanted supernatant and poor separation of phases, are avoided in this new procedure. The water hydration measurement technique was more time-consuming; however it provided a much more accurate comparison of the relative degree of interaction with water of a wide range of products.

The water hydration and water holding capacities of the nine products are presented in Table V. It is of interest to note that agreement between water holding and water hydration capacities was satisfactory only for soybean control. In this sample, the protein solubility was low and phase separation was good, thus the errors in the water holding measurement technique were minimized. For all other products, water holding capacity substantially underestimated the true interaction of water with the product, more accurately described by the water hydration capacity.

Table V. Mean values of water hydration and water holding capacities of canola, sunflower and soybean products (n = 2).

Product		Water hydration capacity (g H_2O/g product)	Water holding capacity (g H_2O/g product)
Canola	- Control	1.8[a] [1] (.100)[2]	0.60[a] (.117)
	- Trypsin	2.3[b] (.080)	0.67[a] (.0095)
	- Linoleate	3.0[c] (.140)	2.02[c] (.050)
Sunflower	- Control	2.9[d] (.190)	1.51[b] (.040)
	- Trypsin	3.2[e] (.075)	1.30[b] (.030)
	- Linoleate	3.8[f] (.125)	0.84[a] (.0065)
Soybean	- Control	3.4[c] (.105)	3.31[d] (.105)
	- Trypsin	2.7[g] (.125)	2.04[c] (.150)
	- Linoleate	4.9[h] (.100)	2.42[e] (.015)

[1]Means in a column sharing the same superscript are not significantly different ($p>0.05$) as determined by Duncan's multiple range test.

[2]Standard error of the mean

To evaluate the effects of source and treatment on the degree of interaction of water with the products, only water hydration capacity values were considered. The control products had significantly different water hydration capacities, with canola having the lowest value (1.8 g H_2O/g product) and soybean the highest (3.4 g H_2O/g product). Quinn and Paton (1979) measured the water hydration capacity of Promosoy 100 and found it to be 3.00 g H_2O/g product. The higher pH of the aqueous dispersions prepared in the present study (pH 7.7 vs pH 7.0) would result in stronger interaction of water with the product. The effect of carbohydrate content appeared to be playing a role in determining WHC, with the high carbohydrate products (soy and sunflower) interacting more strongly with water than the low carbohydrate canola isolate. Fiber residues are well known to have very high water holding capacities, up to 100 g H_2O/g db (Lapsley, 1980). Therefore, small amounts of crude fiber in the products would be expected to strongly influence their water hydration capacities.

Trypsin treatment caused the water hydration capacities to increase in the canola and sunflower products and decrease in the soy product as compared to controls. The increased exposure of charged and polar amino acid groups to the aqueous environment would be expected to increase the interaction of water with the product resulting in increased water hydration capacity, similar to the effect observed on solubility. However, the packing arrangement of protein aggregates and cell wall fragments in the hydrated pellet will also influence the amount of water that can be entrapped by the product. Possibly, the soybean trypsin particles can assume a closer packing arrangement than in the control sample, limiting the water hydration capacity.

Treatment with linoleate resulted in increased water hydration capacity, regardless of product source. Several factors may be responsible for this effect. First, there is the disruption of protein-protein hydrophobic bonds described by Canella et al. (1979) which would expose more polar and ionic groups to the water. Second, the interactions between protein and linoleate would increase the number of negatively charged sites on the protein for hydrogen bonding (Nakai et al., 1980b). Third, the effect of linoleate on aggregation may also be important. Linoleate treatment was observed to increase the mean particle size in the canola and soybean dispersions. Chou and Morr (1979) reported that aggregation may increase water-protein interactions by forming a structural network capable of imbibing water.

5. Rheological Properties

a. Steady shear

The flow behavior rheograms of the nine sample dispersions are presented in Figure 26. All dispersions were found to behave as non-Newtonian pseudoplastic fluids as indicated by the negative rheogram slopes. Sunflower and soy dispersions were higher in viscosity than canola dispersions. The insoluble irregularly shaped carbohydrate and protein materials in the concentrate dispersions offered considerable resistance to flow. As shear rate increased, the particles aligned themselves with the shear planes, offering less resistance to flow and exhibiting a decreased viscosity. Canola dispersions were generally less pseudoplastic than soy and sunflower samples. The spherically shaped protein aggregates offered little resistance to flow at low shear rates and did not exhibit as great a thinning effect as the shear rate was increased, due to the relatively uniform shape. None of the disperions were found to exhibit measurable yield stress values.

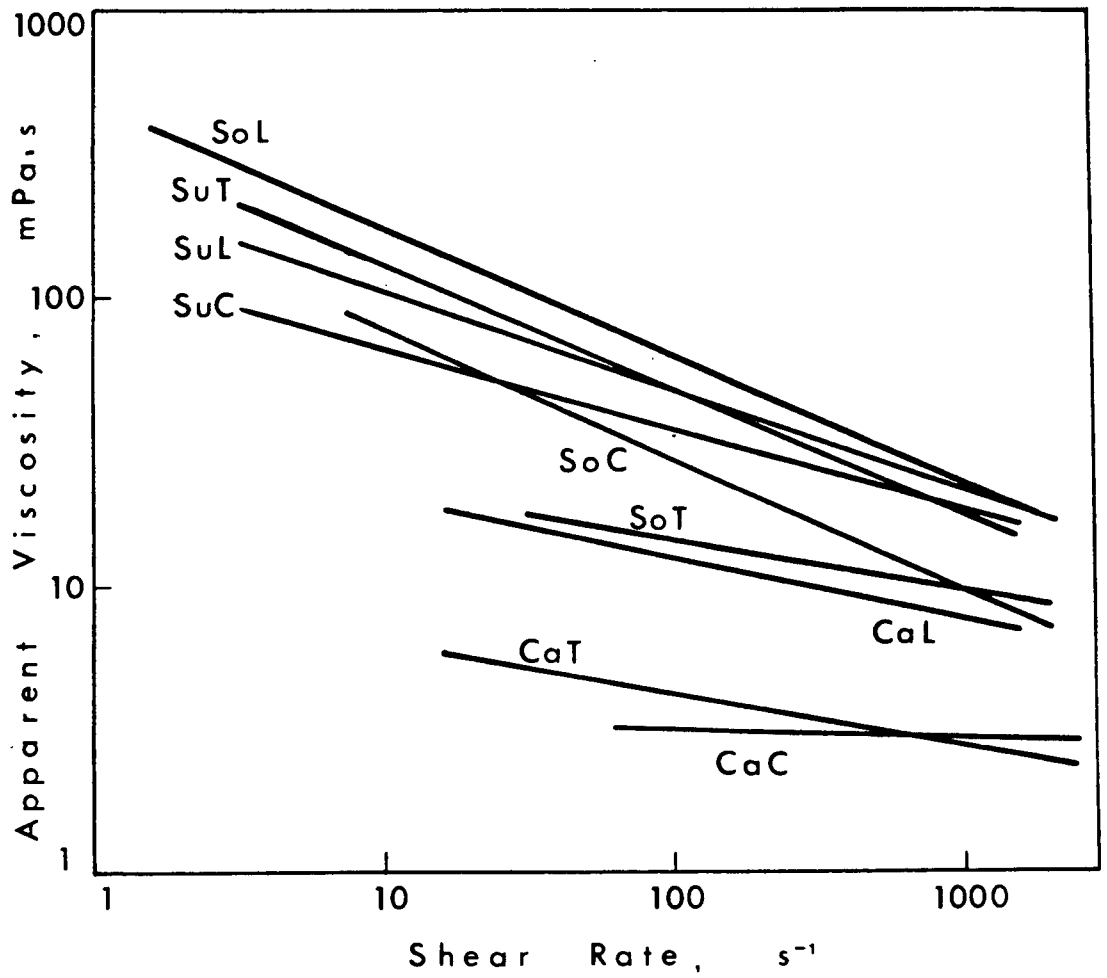

Figure 26. Flow behavior rheograms of untreated (C), trypsin (T) and linoleate (L) treated canola (Ca), sunflower (Su) and soybean (So) products in 10% aqueous dispersions at 20°C.

The Power-law flow parameters are listed in Table VI. The Power-law model fitted the data very well, as indicated by the high values for the coefficient of determination. The degree of pseudoplasticity was indicated by the amount of deviation from a value of 1.0 for the flow behavior index. Values of apparent viscosity at 100 s^{-1} represent apparent viscosities at an intermediate shear rate in the range used in this study. The samples were found to be not significantly thixotropic, as indicated by the lack of a hysteresis loop in the rheograms.

Linoleate treatment brought about an increase in apparent viscosity at a shear rate of 100 s^{-1} and a decrease in flow behavior index in all sources as compared to controls. The effect of trypsin treatment on the viscous properties of the disperisons varied considerably with the source. Trypsin hydrolysis increased the apparent viscosities at 100 s^{-1} and decreased the flow behavior indices of canola and sunflower products. Soy product which had been treated with the enzyme, formed dispersions with lower apparent viscosities at 100 s^{-1} and higher flow behavior indices. These results are in agreement with those of Lynch et al. (1977) who found a decrease in apparent viscosity of a soy protein isolate dispersion as a result of pepsin hydrolysis.

Trypsin treatment was also found to affect the water hydration capacity of soy product differently than canola and sunflower sources. These observations suggest that treatment with trypsin was altering the basic physical and chemical properties of the products in different ways.

Changes in the two phases of the dispersion can be related to the treatment effects on flow behavior. The properties of the aqueous phase may be influenced by the treatments. In pure protein solutions, both apparent

Table VI. Mean values of steady shear Power-law flow parameters of 10% aqueous dispersions of canola, sunflower and soybean products (n = 2).

Product		Flow behavior index, n	Consistency coefficient, m (Pa s^n)	Apparent viscosity at 100 s^{-1} (mPa s)	Coefficient of determination, r^2
Canola	- Control	.955[a] [1] (.0365)[2]	0.0385[a] (.00651)	3.09[a] (.0120)	.941
	- Trypsin	.822[b] (.0125)	0.0950[b] (.00699)	4.16[b] (.065)	.993
	- Linoleate	.788[bc] (.0100)	0.317[c] (.0224)	11.9[c] (.300)	.971
Sunflower	- Control	.729[cd] (.0100)	1.21[d] (.0790)	35.0[d] (3.71)	.989
	- Trypsin	.584[e] (.0160)	3.45[ef] (.517)	50.3[e] (3.91)	.980
	- Linoleate	.663[d] (.0200)	2.26[fg] (.0080)	48.1[e] (4.25)	.977
Soybean	- Control	.577[e] (.0428)	2.19[g] (.584)	29.1[d] (2.47)	.911
	- Trypsin	.828[b] (.0195)	.320[c] (.0491	14.3[c] (.919)	.969
	- Linoleate	.568[e] (.0045)	4.63[e] (.308)	63.0[f] (2.90)	.975

[1]Means in a column sharing the same superscript are not significantly different ($p>0.05$) as determined by Duncan's multiple range test.

[2]Standard error of the mean

viscosity and pseudoplasticity increase as protein concentration increases (Pradipasena and Rha, 1977). Thus, treatments which increase the solubility of the protein in the products might be expected to produce dispersions with increased viscosities and decreased flow behavior indices. Such was found to be the case for canola and sunflower sources, however results for soybean are inconsistent with this explanation. Although an increase in solubility was achieved, effects on flow behavior were observed to be opposite to those expected. Obviously some factor other than solubility was playing a role in determining flow behavior.

The properties of the dispersed phase are also important in determining flow properties. The size, shape and hydrodynamic properties of the colloidal particles influence viscosity. Lee and Rha (1979) compared the flow behavior and particle size ranges of two soy protein isolate dispersions prepared using different mixing techniques. They found that the viscosity of a dispersion containing large particles (100 to 500 μm) was higher than the viscosity of a smaller particle (2 to 20 μm) dispersion.

The shape of the particles is another important factor in determining how easily the particles can move past one another in the dispersion. In general, at the molecular level, long polymers offer greater resistance to flow than the same concentration of smaller, more symmetrical molecules. For larger complex aggregates, hydration and swelling become important factors, affecting particle size and density and immobilizing some volume of free water. Intermolecular interactions also play a role in governing flow properties. Aggregation of particles may increase to such an extent that the dispersed phase may sediment. However, if the degree of interaction is fairly low, increases in particle size, hydration and swelling may lead to the formation

of stable high viscosity dispersions. The effects of trypsin hydrolysis on the flow behavior of the soy protein concentrate may be related to changes in the properties of the dispersed phase, which could outweigh the effect of increased solubility on the aqueous phase.

In the case of linoleate treatment, also, the effects on flow behavior cannot be attributed entirely to the effect of increased solubility. Linoleate treatment of the canola isolate was unsuccessful in increasing protein solubility and therefore some other factor must be contributing to the observed increase in apparent viscosity at 100 s^{-1}, and pseudoplasticity of the dispersion. The increase in aggregate size, observed under the light microscope, accompanied by increased hydration, may be at least partially responsible.

b. Dynamic shear behavior (viscoelasticity)

The viscoelastic properties of the nine product dispersions were evaluated at 20, 45, 70 and 95°C. The elastic and viscous moduli of a linearly viscoelastic fluid are independent of the amplitude and duration of oscillation and dependent on the oscillatory frequency. To determine the relationship between the viscoelastic parameters and frequency, dynamic testing was carried out at five different frequencies in the first experimental run. The results indicated that a very poor relationship exists for these samples. Figures 27 and 28 show the recorded values of storage modulus and loss tangent as functions of frequency, at 45°C. Similar results were obtained at 20, 70 and 95°C. Generally, increases in storage modulus and decreases in loss tangent are expected to occur as frequency increases.

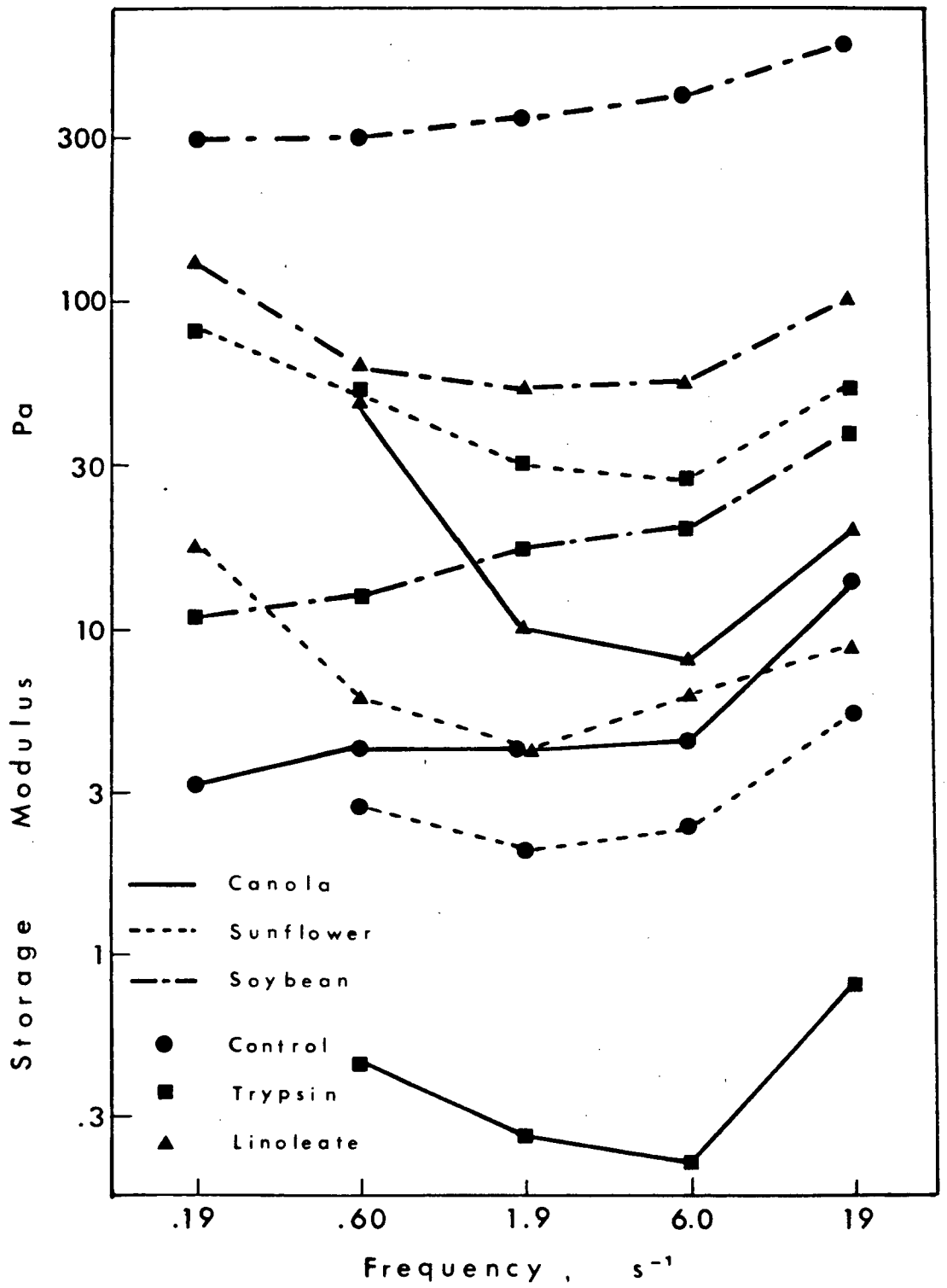

Figure 27. Effect of frequency on storage moduli of 10% product dispersions at 45°C.

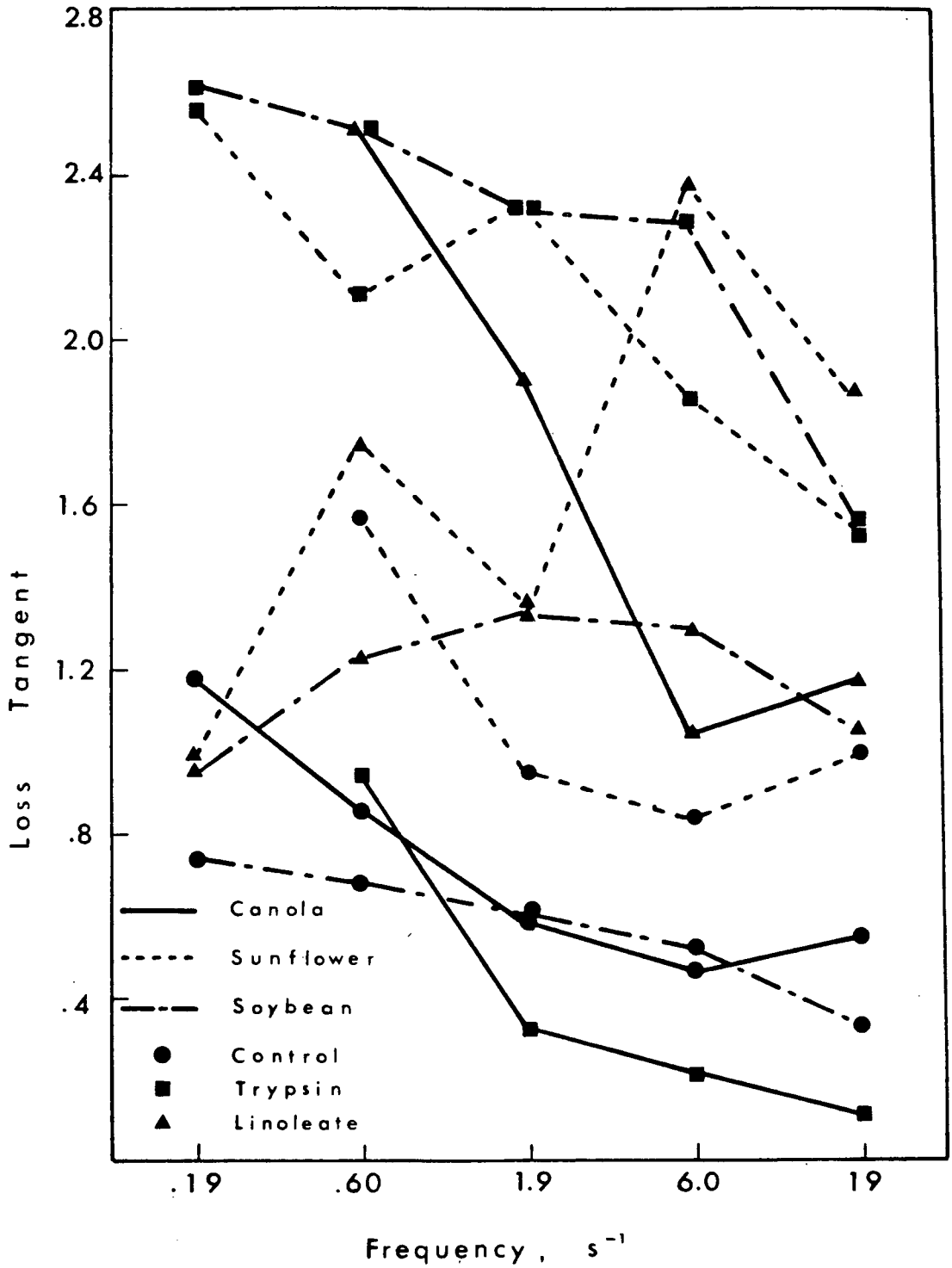

Figure 28. Effect of frequency on loss tangents of 10% product dispersions at 45°C.

The values of the viscoelastic parameters were also found to fluctuate over the duration of testing at a constant frequency. Some form of time-dependence was concluded to be interfering with the expected behavior. Therefore, only values recorded at a frequency of 6.0 s^{-1}, taken soon after initiation of oscillatory testing were examined to assess the effects of treatment on the viscoelastic properties. For the replication of the experiment, only a frequency of 6.0 s^{-1} was employed.

Table VII presents the values of storage modulus and loss tangent obtained for the nine samples at 20°C and 6.0 s^{-1}. The high degree of variability as indicated by the large values of standard error of the mean prevented meaningful statistical analysis of this data. The high variability of the replicate samples can be attributed to dehydration of the sample at the circumference of the fixture and interference by particles that were relatively large in relation to the gap thickness. In future work these sources of error may be minimized by using plate/plate fixtures with a large separation rather than a cone/plate system, and enclosing the fixtures in a high humidity chamber.

The effects of treatment on the viscoelastic properties of the dispersions at 20°C can be interpreted from trends in the data (Table VII). The soy control dispersion exhibited the highest value of storage modulus, likely as a result of the large amount of insoluble carbohydrate and protein material. The storage modulus describes the elasticity of the sample. Results for all trypsin-hydrolyzed samples suggested a trend toward lower elasticity, due perhaps to increased solubility and reduced aggregate size. The linoleate effect was less distinct. For canola, an increase in elasticity was observed, probably as a result of larger aggregate size. Soy and sunflower products,

Table VII. Mean values of viscoelastic properties of 10% aqueous dispersions of canola, sunflower and soybean products at 20°C (n = 2).

Product		Storage modulus (Pa)	Loss tangent
Canola	- Control	1.39 (0.478)[1]	1.12 (0.160)
	- Trypsin	0.202 (0.116)	4.25 (3.90)
	- Linoleate	4.60 (3.20)	1.14 (0.670)
Sunflower	- Control	3.94 (2.79)	2.76 (1.06)
	- Trypsin	2.07 (0.295)	2.51 (1.65)
	- Linoleate	2.60 (1.39)	2.89 (1.57)
Soybean	- Control	55.2 (20.7)	1.46 (0.125)
	- Trypsin	1.85 (1.14)	2.60 (2.19)
	- Linoleate	30.2 (21.4)	1.37 (0.155)

[1]Standard error of the mean.

treated with linoleate, exhibited a decrease in elasticity over controls, possibly related to increased solubility.

The values of loss tangent (Table VII) reflect the relative proportions of the elastic and viscous natures of the material. Loss tangents greater than 1.00 indicate behavior that is predominantly viscous and values less than 1.00 describe predominantly elastic material. At 20°C, all dispersions exhibited viscoelastic behavior that was more viscous than elastic. The higher loss tangent values reported for trypsin-hydrolyzed soy and canola samples indicate viscoelastic behavior that is more viscous in nature than corresponding control and linoleate dispersions. This behavior was also reflected in the low values of storage modulus for these trypsin-treated samples. Linoleate treatment did not appear to affect the loss tangents measured for the dispersions, as compared to controls.

The effect of temperature on the viscoelastic properties of the dispersions is shown in Figures 29 and 30. As the temperature increased, the storage moduli (Figure 29) of the dispersions generally increased. This would be caused by the formation of a gel-like matrix within the sample, capable of storing more of the deformation energy. The values of loss tangent (Figure 30) were observed to follow a decreasing trend as temperature increased. This reflects a shift in the ratio of viscous to elastic natures, as the dispersions undergo network formation to produce semi-solid gel-like materials. The effect of source and treatment on the response of the dispersions to heating cannot be discerned from the results due to the high degree of variability of the data.

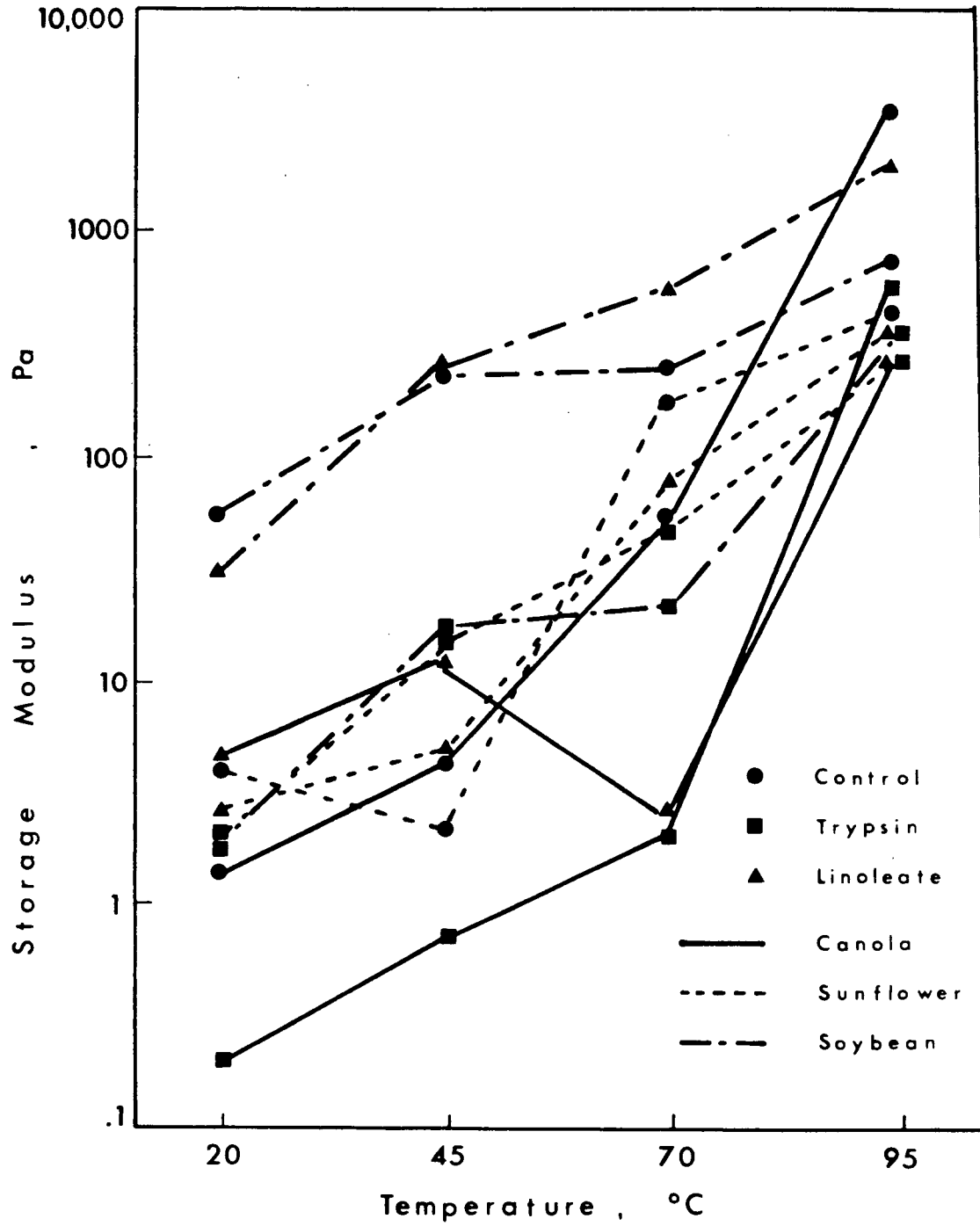

Figure 29. Effect of temperature on storage moduli of 10% product dispersions at a frequency of 6 s^{-1}.

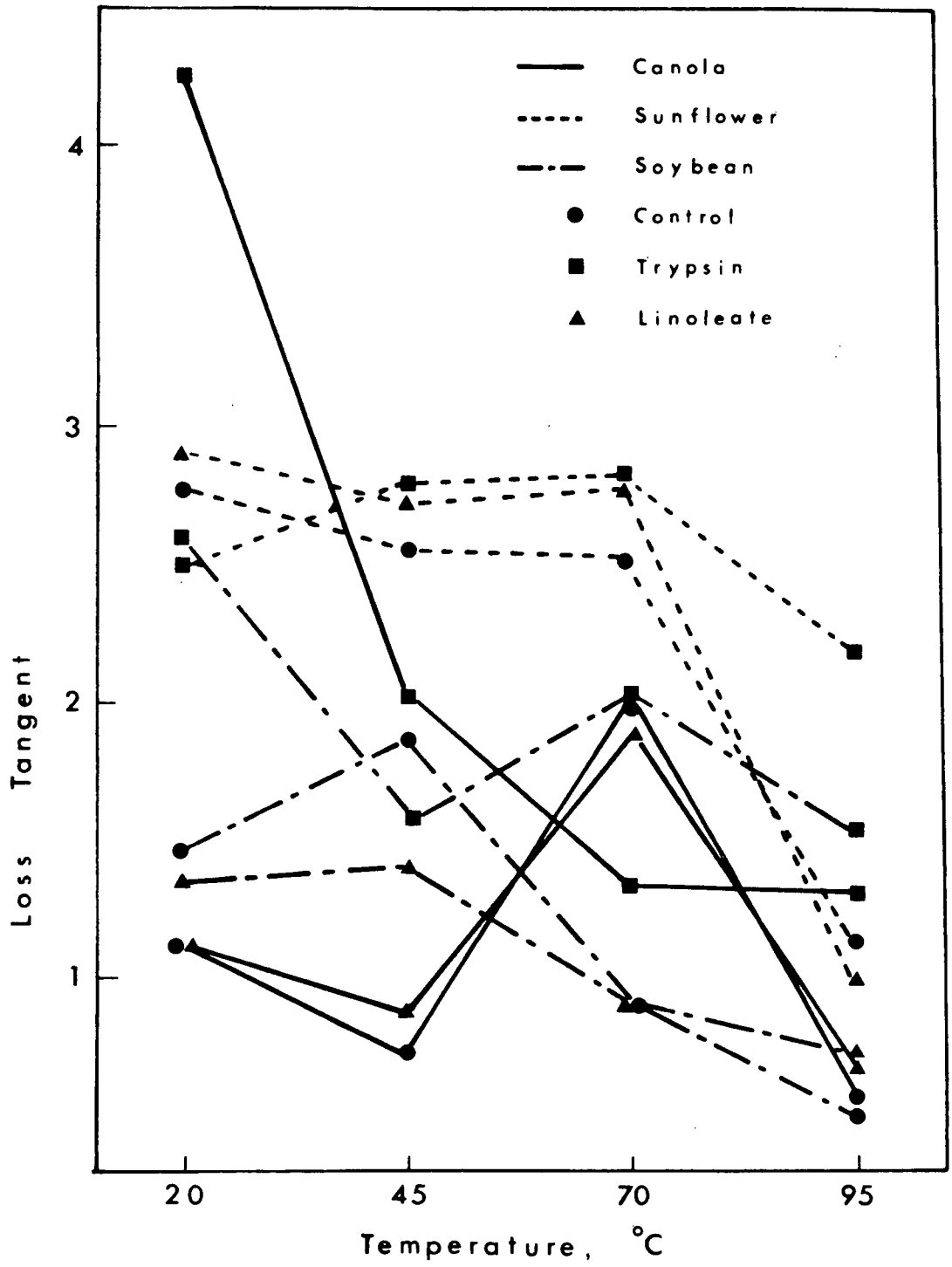

Figure 30. Effect of temperature on loss tangents of 10% product dispersions at a frequency of 6 s^{-1}.

D. Statistical Analysis

1. Analysis of variance

The data collected for 10 measured variables were analyzed as a 3 x 3 factorial analysis of variance, to determine the significance of the observed effects of "source" and "treatment". The results are presented in Table VIII. Source and treatment were found to significantly affect most of the measured properties. In cases where the interaction between source and treatment is not significant, methods can be employed to determine the effects of a particular source or treatment. Duncan's multiple range test, at the 5% level of significance, was employed for this purpose. For example, soy and sunflower products have significantly different protein contents from canola products, regardless of the treatment (Table IX). Also the protein contents of control and trypsin-treated products differ significantly from those of products treated with linoleate, irrespective of source. For the measured properties for which the interaction term was significant, it was impossible to separate the effect of source from the treatment effect. Therefore, single factor analyses of variance were also was performed on the data to determine the significance of the effect of "product" on the measured properties. Duncan's multiple range tests, at the 5% level of significance were performed to determine differences among individual means, resulting in the superscripts assigned to means recorded in Tables II-VII.

2. Simple correlations among variables

Correlation coefficients calculated for correlations among 12 measured variables are reported in Table X. It is of interest to note that solubility, the functional property which is most commonly reported in the literature, was

Table VIII. Levels of significance of F-values calculated from data collected for each measured variable by a 3 x 3 (source x treatment) factorial analysis of variance.

Variable	Source df = 2	Treatment df = 2	Interaction df = 4
Protein	**	**	NS
Carbohydrate	**	NS	NS
Moisture	**	*	**
pH	**	**	**
Conductance	**	**	**
Solubility	**	**	**
Water hydration capacity	**	**	**
Flow behavior index	**	**	**
Consistency coefficient (log)	**	**	**
Apparent viscosity at 100 s^{-1} (log)	**	**	**

** $p \leq 0.01$ * $p \leq 0.05$ NS $p > 0.05$

Table IX. Results of Duncan's Multiple Range testing for variables for which the source x treatment interaction term was not significant ($p \geq 0.05$).

Variable			
Protein	Canola	Sunflower	Soybean
	Control	Trypsin	Linoleate
Carbohydrate	Canola	Sunflower	Soybean

Source or treatments sharing the same underscore are not significantly different ($p > 0.05$).

Table X. Correlation coefficients calculated for linear correlations among all measured variables (n = 9)

	Protein	Carbo-hydrate	Solubility	Water hydration	Water holding	n value
Conductance	-.059	-.249	.186	.301	.306	.040
pH	.490	-.626	.015	-.295	-.418	.363
Loss tangent (20°C)	.173	-.001	.387	-.215	-.493	.071
Storage modulus (20°C)	-.502	.462	-.542	.503	.836	-.605
Apparent viscosity (100 s^{-1})	-.819	.667	.573	.878	.268	-.865
m value	-.756	.605	.429	.874	.356	-.886
n value	.767	.670	-.194	-.836	-.553	
Water holding capacity	-.615	.566	-.545	.508		
Water hydration capacity	-.858	.573	.370		.950	Apparent viscosity
Solubility	-.231	.084		.311	.456	Storage modulus
Carbohydrate	.893		-.442	-.086	-.192	Loss tangent
		-.465	-.285	-.235	-.230	pH
	-.025	-.317	.272	-.113	.076	Conduc-tance

	pH	Loss tangent	Storage modulus	Apparent viscosity	m value

$r_{.05}$ = .666 $r_{.01}$ = .798

found to be not significantly correlated ($p>0.05$) with any of the other measured parameters. The non-significant correlation between water holding and water hydration capacities confirmed the earlier observation that the water holding capacity technique is not a meaningful measure of the hydration abilities of a product. Some of the more interesting significant correlations were those involving water hydration capacity and apparent viscosity (at 100 s^{-1}), as these functional properties relate closely to the performance of the products in food systems. Figures 31 to 33 present graphically, some of these correlations.

When plotted, the correlation between consistency coefficient (m) and flow behavior index (n) appeared to follow a semi-logarithmic relationship, in spite of the high value of the correlation coefficient calculated for the linear model. The data were re-plotted as log m vs. n (Figure 34) and an even higher value of the correlation coefficient ($r = .958$) was computed for this semi-log correlation. Lefebvre and Sherman (1977) also report a highly significant correlation ($r = 0.958$, $p\leq0.01$) between log m and n based on results from several different sunflower isolate dispersions.

Neither pH nor conductance were found to be correlated with any of the other measured properties, indicating that the different pH and ionic strengths of the product dispersions was not solely responsible for the observed treatment effects.

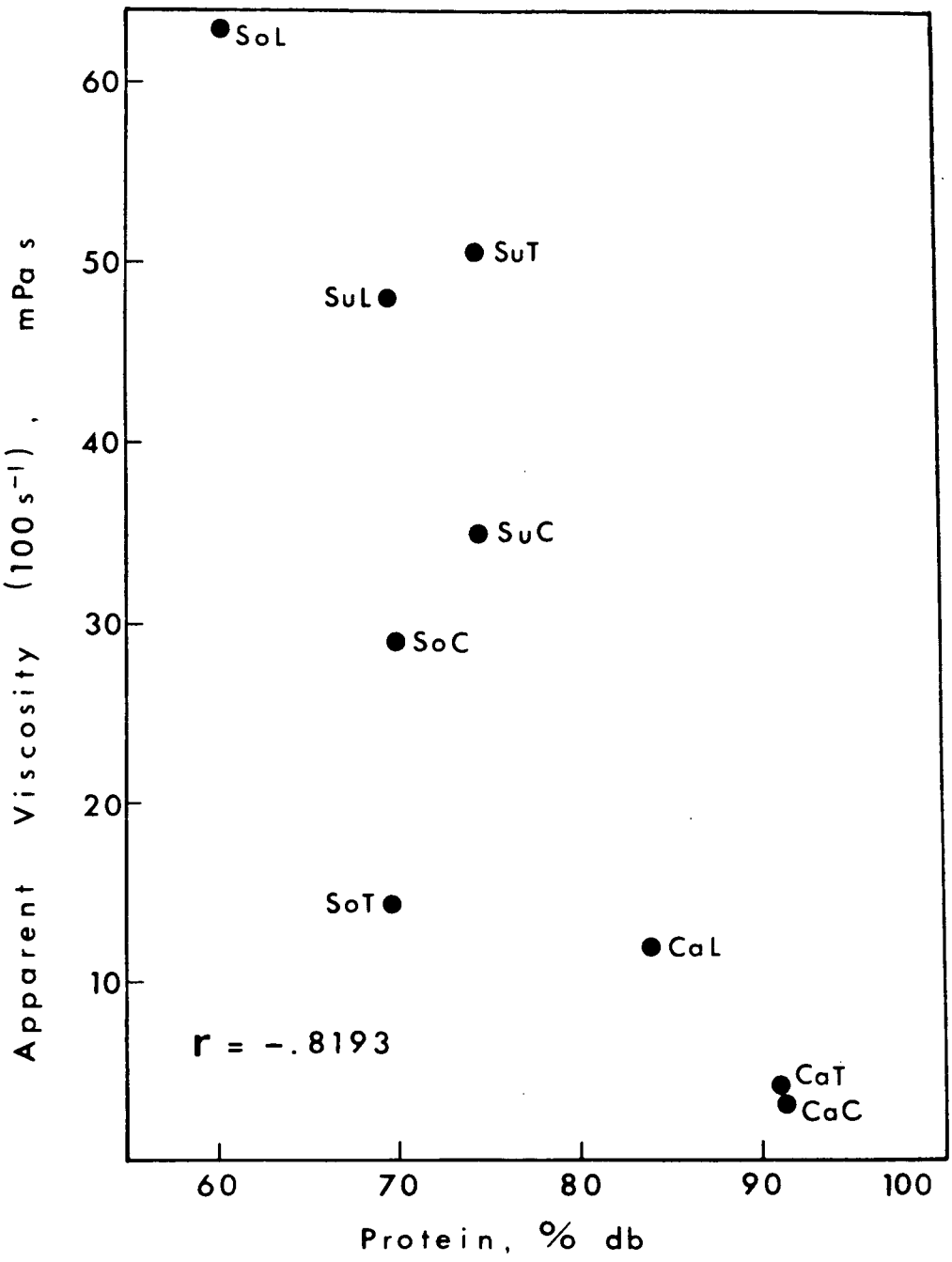

Figure 31. Correlation between apparent viscosity (100 s^{-1}) of 10% product dispersions at 20°C and protein content of untreated (C), trypsin (T) and linoleate (L) treated canola (Ca), sunflower (Su) and soybean (So) products.

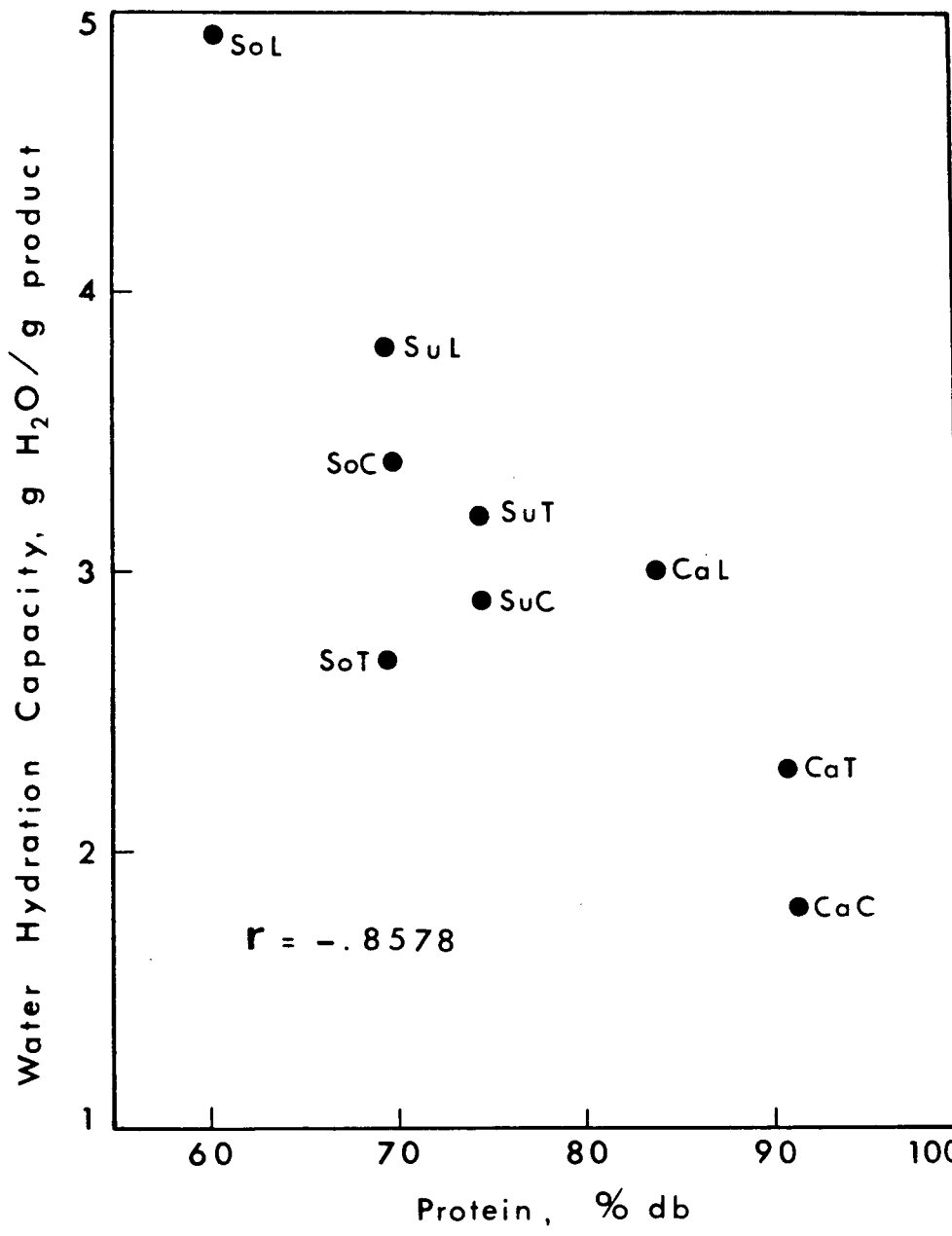

Figure 32. Correlation between water hydration capacity and protein content of untreated (C), trypsin (T) and linoleate (L) treated canola (Ca), sunflower (Su) and soybean (So) products.

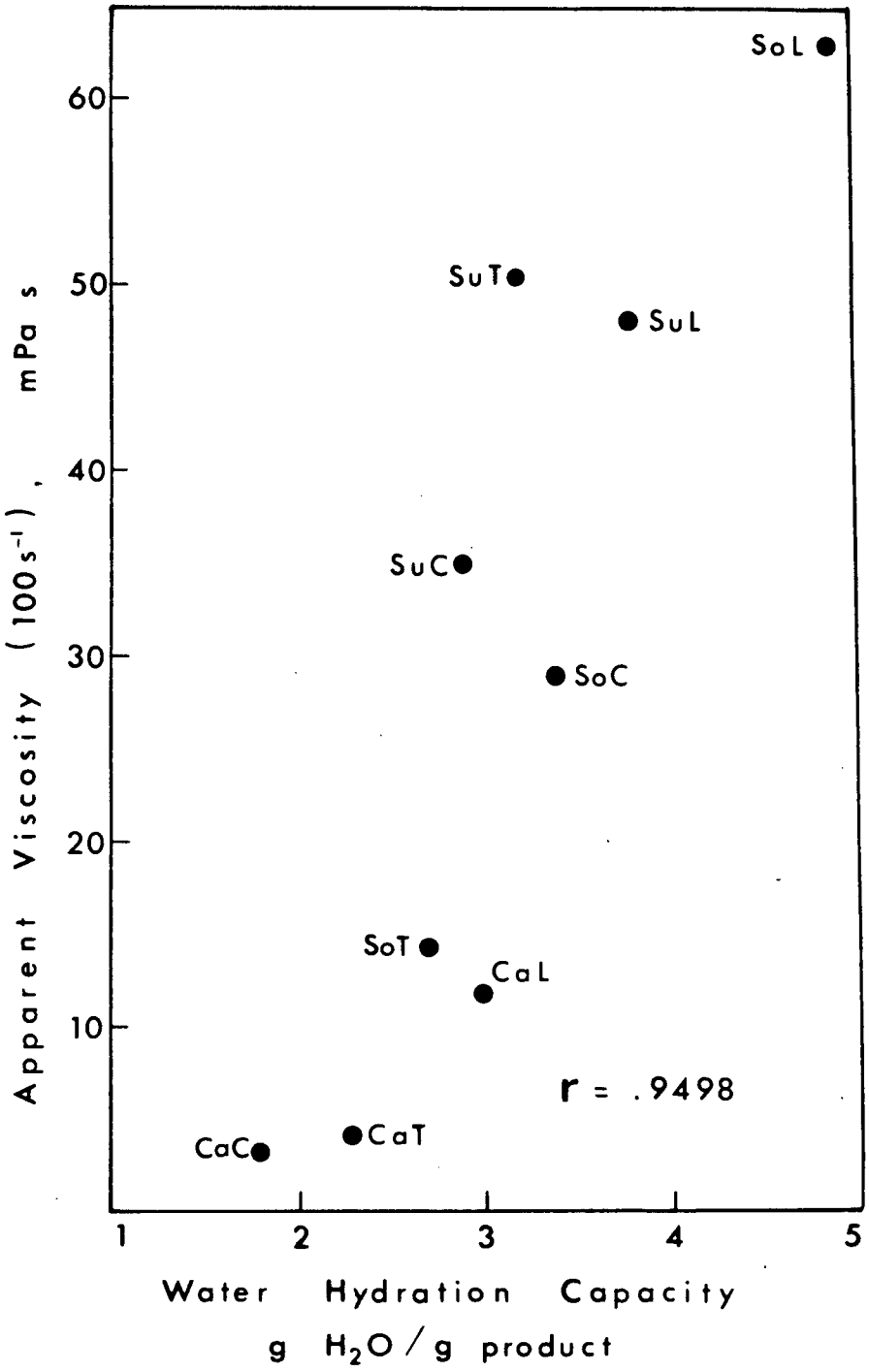

Figure 33. Correlation between apparent viscosity (100 s^{-1}) of 10% product dispersions at 20°C and water hydration capacity of untreated (C), trypsin (T) and linoleate (L) treated canola (Ca), sunflower (Su) and soybean (So) products.

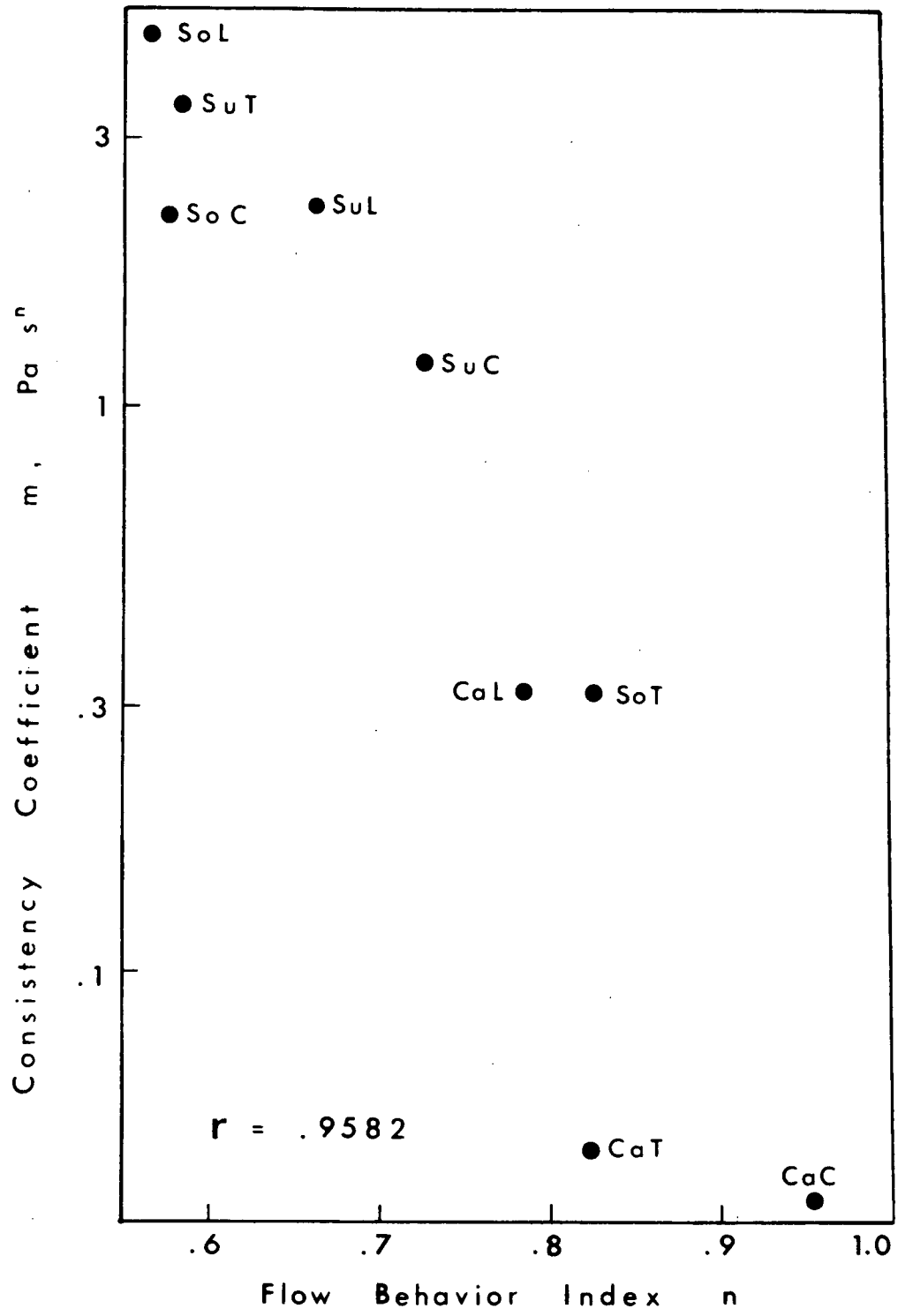

Figure 34. Correlation between consistency coefficient (m) and flow behavior index (n) for 10% dispersions of untreated (C), trypsin (T) and linoleate (L) treated canola (Ca), sunflower (Su) and soybean (So) products at 20°C.

SUMMARY AND CONCLUSIONS

Nine high-protein oilseed products were examined for compositional, structural and functional properties. Trypsin and linoleate treated products differed significantly from untreated controls in several of the parameters measured.

Trypsin hydrolysis did not affect the protein, carbohydrate and moisture contents of products prepared from canola, sunflower or soybean sources. Under the light microscope, 10% dispersions of trypsin-hydrolyzed canola and soybean products were observed to contain smaller dispersion particles than were found in controls. A change in particle size could not be detected in the trypsin-treated sunflower samples. Treatment with the enzyme appeared to increase the number of sites available for gel network formation, as indicated by examining thermally treated 10% dispersions under the scanning electron microscope. However, the protein-protein interactions were observed to be weak in nature. In the case of trypsin-treated soy product, the material produced by heating a 10% dispersion was very sensitive to mechanical destruction and did not possess the necessary structural integrity to permit microscopic observation.

Measurements of conductance of 10% aqueous dispersions, recorded as an index of ionic strength, indicated that trypsin-hydrolyzed samples had higher ionic strengths than controls. Protein solubility was found to increase as a result of trypsin treatment. Canola and sunflower products treated with trypsin exhibited higher water hydration capacities, while trypsin-treated soy samples were found to have lower hydration abilities.

Trypsin-treatment produced canola and sunflower dispersions with lower flow behavior indices and higher values of apparent viscosity at 100 s^{-1}, as compared to controls. Soy product which had been similarly treated demonstrated the opposite effect, with higher flow behavior indices and lower apparent viscosities at 100 s^{-1}. Although significant differences could not be detected, trypsin-hydrolyzed products in 10% aqueous dispersion exhibited trends toward lower values of storage modulus and higher loss tangents. These trends reflected viscoelastic behavior that more closely describes ideal viscous fluids than did the behavior of corresponding controls.

Treatment of the oilseed products with linoleic acid brought about significant decreases in protein and carbohydrate contents, due to displacement of some weight of these components by the linoleic acid which was added at a level of 8.7%. Observed differences in moisture content were not attributed to treatment effects. Light microscopic observation of 10% dispersions of linoleate-treated products revealed the presence of larger protein aggregates in canola and soybean sources as compared to controls. The structural properties of the sunflower dispersions were found to be unaffected by linoleate treatment. Linoleic acid treatment was observed to increase protein-protein interactions in thermally treated 10% dispersions from all sources, resulting in stronger gel-like materials than were produced by heating control dispersions.

The ionic strengths of 10% product dispersions were found to increase as a result of linoleate treatment, as indicated by conductance measurements. Protein in linoleate-treated sunflower and soy samples was more soluble than in untreated products, while canola products exhibited no change in protein

solubility as a result of linoleate treatment. Higher values of water hydration capacity were recorded for all linoleate-treated products as compared to controls.

Dispersions of linoleate-treated products were found to have significantly higher apparent viscosities at 100 s^{-1} than control dispersions. The flow behavior index of the 10% canola product dispersion was reduced significantly as a result of linoleate treatment. Similarly treated soy and sunflower product dispersions exhibited trends toward lower values of flow behavior index. Effects of linoleate treatment on the viscoelastic properties of 10% dispersions could not be discerned from the data.

The water hydration capacity technique proposed recently by Quinn and Paton (1979) was found to provide a meaningful measurement of the hydration abilities of dry powdered protein products.

Viscoelastic properties of 10% product dispersions were recorded at 20, 45, 70 and 95^{o}C to follow the changes occurring during the gelation process. Trends toward increases in storage moduli and decreases in loss tangents with increasing temperature were observed.

This research has confirmed the potential of modifying agents for use in producing protein-rich products with desired functional properties. Trypsin and linoleic acid treatments brought about functionality changes in aqueous systems that could be attributed not only to the degree of interaction of the product with water, but also to the size and shape of the product particles. It has been demonstrated that complex relationships exist among the functional, structural and compositional properties of oilseed concentrates and isolates. The results provide an important basis for further research to define clearly the physico-chemical bases of functional properties. As long as food

scientists utilize functionality tests to evaluate the potential behavior of protein products in food systems, it will be necessary to continue to improve our understanding of the basic principles governing these phenomena.

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