ISOLATION AND CHARACTERIZATION OF SOYBEAN PROTEIN AND WHEY PROTEIN CO-PRECIPITATES

By

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"To My Father and My Mother My Password for Success"

"To My Sisters, My Brothers and Friends Source of My love"

ABSTRACT

Protein co-precipitates were prepared from whey powder and soybean flour using various extraction and co-precipitation techniques. The effect of extraction and co-precipitation on co-precipitate yield was investigated. Native and sodium dodecyl sulfate polyacrylamide gel electrophoresis (Native-PAGE, SDS-PAGE) and light compound microscopy (LCM) were used to study the structure of the co-precipitates. The rheological and gelation properties of the co-precipitates were determined. Highest yield (45%) for NaOH/Isoelectric Point IEP-Heating-Cooling. co-precipitate was obtained using the following conditions of extraction; extraction temperature, 40°C; temperature of precipitation 95°C, and pH of precipitation was 4.5. The yield of co-precipitates was affected by chelating agents and pH of precipitation and temperature of precipitation. Native-PAGE showed that 2 new protein bands result from the interactions between whey and soybean proteins during preparation of the co-precipitate. SDS-PAGE showed that the new proteins dissociated to give the protein subunits of whey and soybean proteins. LCM results showed differences in microscopic structure between the whey and soybean protein precipitates and the protein co-precipitates. Gels were characterized by measurement of water holding capacity (WHC), gelation start temperature (GST) and denaturation start temperature (DST) and gel strength (GS). Gels (16%) from a protein co-precipitate Mixed Powder MP:NaOH/IEP-Cooling had higher WHC and GS than gels from whey protein precipitate, soybean protein precipitate and protein co-precipitates Mixed Extract ME:NaOH/IEP-Cooling and co-precipitates MP: and ME:NaOH/IEP-Heating-Cooling. The DST of protein co-precipitates was dependent on protein concentration and pH, while GST was relatively dependent on protein concentration.

RÉSUMÉ

Des co-précipités de protéines ont été préparé à partir de poudre de petit-lait et de farine de soja en utilisant diverses techniques d'extraction et de co-précipitation. L'effet d'extraction et de co-précipitation sur le rendement du co-précipité a été examiné. Une électrophorèse sur gel natif et de dodecyl sulfate sodium (native PAGE, SDS-PAGE) ainsi que la microscopie à lumière composée ont été utilisés pour étudier les co-précipités. Les propriétés rhéologiques et de gélification des co-précipités ont été déterminées. Le plus haut rendement (45 %) pour le co-précipité NaOH/IEP-Chauffant - Refroidissant a été obtenu en utilisant les conditions d'extraction suivantes : température d'extraction de 40 °C, rapport petit lait/soja de 5/10, pH d'extraction de 11, température de précipitation de 95°C, et un pH de précipitation de 4.5. Le rendement des co-précipités a été affecté par des agents chélateurs, le pH du IEP et la température de précipitation. La Native-PAGE a montré que 2 nouvelles protéines résultent des interactions entre les protéines de petit-lait et de soja durant la préparation des co-précipités. La SDS- PAGE a montré que les nouvelles protéines se sont dissociées pour donner des sous-unités de protéines de petit-lait et de soja. La microscopie à lumière composée souligne les différences dans la structure microscopique entre les précipités de protéines de petit lait et de soja et les co-précipités de protéines. Les gels ont été caractérisés par la mesure de la capacité de rétention en eau (CRE), de la température de commencement de dénaturation et de gélification et de la fermeté du gel. Les gels (16 %) de co-précipités protéiques de MP : préparés par NaOH/IEP-Refroidissant et les co-précipités de MP avaient une plus haute CRE et une plus haute fermeté que les gels de précipité de protéine de petit lait, le précipité de protéine de soja et le co- précipité

protéique MP : préparé par NaOH/IEP-Refroidissant et les co-précipités MP : et ME : NaOH/IEP-Chauffant –Refroidissant. La température de commencement de dénaturation du co-précipité protéique dépend plus de la concentration et du pH, alors que la température de gélification est relativement dépendante de la concentration et du pH.

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ABBREVATIONS

α–la:	α-lactalbumin
β-lg:	β-lactoglobulin
BSA:	Bovin Serum Albumin
Da:	Dalton
DST:	Denaturation Start Temperature
EDTA:	Ethylene Diamine Tetra Acetic acid
G':	Storage Modulus
G":	Loss Modulus
GST:	Gelation Start Temperature
IEP:	Iso Electric Point
KDa:	Kilodalton
LCM:	Light Compound Microscopy
ME:	Mixed Whey extract and Soybean extract
MP::	Mixed Whey powder and Soybean flour
MW:	Molecular Weight

Na-HEXA:	Sodium Hexametaphosphate
NaOH/Cooling:	Sodium Hydroxide Extraction/Cooling Precipitation
NaOH/IEP-Cooling:	Sodium Hydroxide Extraction/IEP and Cooling Precipitation
NaOH/IEP-Heating-Cooling:	Sodium Hydroxide Extraction/IEP, Heating and Cooling Precipitation
NaCl-NaOH/Cooling:	Sodium Hydroxide and 10%NaCl Extraction/Cooling Precipitation
NaCl-NaOH/IEP-Cooling:	Sodium Hydroxide and 10%NaCL Extraction/IEP and Cooling Precipitation
NaOH-Papain/Cooling:	Sodium Hydroxide and Papain Extraction/Cooling Precipitation
N%:	Nitrogen content%
N:	Nutine
PAGE:	Polyacrylamide Gel Electrophoresis
SDS:	Sodium Dodecyl Sulfate
STD:	Standard
WHC:	Water Holding Capacity
W/V:	Weight/Volume

CHAPTER 1

INTRODUCTION

Advances in the technology of protein production in the past two decades have made it possible to produce different types of proteins commercially from various sources of raw materials. Many food characteristics, such as appearance, texture, and stability are greatly influenced by the physical and chemical properties of food proteins (Towler, 1970). In addition, increasing world population, rising cost of foods from animal source, and the continuing need to improve the nutritional and functional properties of proteins are contributing to accelerated research on proteins as food ingredients (Bookwalter et al., 1971; Dendy et al., 1975; El-Sayed et al., 1987; Youssef et al., 1995).

Protein co-precipitates may be an alternative and more economical method of preparing extruded food for production of high protein extrudates of desirable functional characteristics (Hagan et al., 1986). Milk and whey protein co-precipitates contain lysine in a relative high concentration, and can be useful for complementing plant proteins, mainly those of cereals (Babella, 1982). Protein co-precipitates utilization can help overcome the pollution problems of cheese whey disposal (Kebray, 1993).

The high nutritional values of the whey proteins, α-lactalbumin and β-lactoglobulin, have been recognized (Forsum, 1975; Loewnstein and Paulraj, 1972). Proteins derived from animal source, such as milk proteins, are expensive and are not readily available in sufficient quantities. Vegetable proteins are cheaper than animal proteins (Pinthong et al., 1980). Some plant proteins lack certain essential amino acids, and this limits their nutritional quality. For example, rice and wheat proteins are deficient in lysine, and soy proteins have low levels of the sulfur containing amino acids. Milk

proteins have relatively high amounts of these amino acids; therefore a complementary nutritional effect can be achieved in protein blends that contain both plant and milk proteins.

The combination of a wide range of physical and functional properties and superior nutritive properties allows co-precipitates to be used as an ingredient in a variety of food products either by contributing necessary functional properties to the ingredient mix or for nutritional purposes.

Several processes have been developed for the manufacture of protein co-precipitates. Montigny (1983) described a process for the precipitation of casein and or whey protein by combined application of acidification and heating to produce a co-precipitate suitable as a pre-cheese for cheese manufacture. An example of the utilization of protein co-precipitates in meat products, the effect of co-precipitates on the quality and yield of boiled sausages (Salavatulina et al., 1983); it is be possible to replace the meat in comminuted foods with mixtures of other proteins such as blood, lupin, sunflower, casein whey and leaf protein concentrate (Geoffrey et al., 1976).

The overall objective of this research was to investigate the extraction and precipitation of protein co-precipitates from whey powder and soybean flour, and to determine the properties of the co-precipitates. The specific objectives were: (i) to prepare protein co-precipitates using different extraction and precipitation techniques, (ii) to investigate the factors and conditions which affect yield of protein co-precipitate, (iii) to use polyacrylamide gel electrophoresis to study the protein profile, and (iv) to investigate the rheological and gelation properties of the protein co-precipitates.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein Co-precipitates

The term protein co-precipitates was first used by Scott (1952) to describe a protein that contains a combination of both the casein and whey proteins derived from milk, by acidification and heat; initially term was confined to the proteins from milk. More recently, the term has broadened to cover combination of proteins from milk as well as other sources of proteins. The initial developments in the production and uses of co-precipitate occurred in the USA and USSR. The process for preparation of protein co-precipitates from rapeseed isolates and concentrates with added whey was referred to as the co-precipitation process (Thompson, 1977). The production of co-precipitates is performed by isoelectric coagulation, precipitation being effected by simultaneous effects of acid and heat combination, or acid, heat and precipitating agents such as $CaCl_2$ (Babella, 1982).

2.2 Types of Protein Co-precipitates

2.2.1 Milk Protein Co-precipitates

Milk co-precipitates are products from whey proteins reacted with caseins and jointly precipitated by heat, calcium addition and pH adjustment. The process for co-precipitation of milk protein was patented in 1952 (Southward and Goldman, 1975). It is known that whey proteins are stabilized against heat precipitation by casein (Rowland, 1937). When whey proteins are denatured in the presence of casein, the exposed

sulphydryl groups of the whey proteins react preferentially with the casein (Creamer et al., 1978). It is possible for the quantity of denatured milk protein co-precipitates to reach 98–100% of the total proteins (Babella, 1982).

2.2.1.1 Preparation of Milk Protein Co-precipitates

Milk protein co-precipitates have been prepared by heating milk or a mixture of milk and other proteins (whey protein and casein) to a temperature above 60°C for a certain period of time (5, 20 and 20 min). After heat treatment, the protein complex can be precipitated directly or it can be cooled and then precipitated. Milk protein co-precipitates are achieved with either acid, divalent ions such as calcium or other ions that affect the solubility of the protein complex (Kosaric and Ng, 1983).

2.2.1.2 Properties of Milk Protein Co-precipitates

2.2.1.2.1 Composition of Milk Protein Co–precipitates

The composition of milk protein co-precipitates is affected by the extent of washing of the precipitated materials (Buchanan et al., 1965). The calcium content of milk protein co-precipitate is determined mainly by the pH of the co-precipitation (Muller et al., 1967). Because they contain substantial portion of whey proteins, milk protein co-precipitates are rich in the sulphur containing amino acids, cysteine and cystine (Southward and Goldman, 1975).

2.2.1.2.2 Physical Properties of Milk Protein Co-precipitates

The general physical properties of milk protein co-precipitates may be regarded in much the same way as the physical properties of the individual proteins when examined alone or dispersed or dissolved in water. These properties include pH, solubility (in water, alkali, or acid), density, particle size, viscosity and color of solution.

The pH of milk protein co-precipitate is affected to some extent by the number of washes in water during the preparation of the product (Southward and Goldman, 1975). The viscosity of solutions of soluble milk protein co-precipitate in water at different concentrations, temperature and shear rates was measured by Hayes et al. (1969). Smith and Snow (1968) and Hayes et al. (1969) reported that the whiteness of milk protein co-precipitates was affected by pH, calcium content, calcium chloride and polyphosphate content of the co-precipitates.

The bulk density of milk protein co-precipitates can vary markedly depending on the method of manufacture. Granular, insoluble co-precipitates have a density of approximately 0.6g/ml, depending on particle size. The density of spray dried soluble co-precipitates can be much lower (Southward and Goldman, 1975). Granular co-precipitates are comparatively coarse and must be ground before they can be used in food products.

2.2.1.2.3 Nutritional Properties of Milk Protein Co-precipitates

The nutritional value of milk protein co-precipitates has been assessed from its amino acid composition, in comparison with that of casein and other reference proteins;

amino acid analysis of milk protein co-precipitates have been reported by Muller et al. (1966), Resmini et al. (1971) and Lohrey et al. (1974).

2.2.1.2.4 Functional Properties of Milk Protein Co-precipitates

Functional properties of proteins (e.g. solubility, emulsifying capacity, foaming characteristics, water holding capacity, geleing capacity) refer to the ability of protein to perform a specific function in food product. The desirability and magnitude of these functions depend on the specific food and its processing and utilization requirement. To be successful in food applications, proteins should possess desirable functional characteristics (Poure-el, 1981). For example, solubility is important in milk beverages, emulsification is important in processed cheese, stability and viscosity are important in fermented milk, and foaming is vital for ice cream (Kinsella, 1982).

Ramshaw and Dunstone (1970) found that the development of "gluey" off-flavors in low calcium co-precipitates was inhibited by the addition of 0.01-0.05% sodium metabisulphate; they reported an improvement in the flavor stability of milk protein co-precipitates by heating them in solution to encourage the formation of volatile off-flavor components that could be removed during drying.

The baked loaves produced from the low absorption milk protein co-precipitates have been shown to be more acceptable than those produced from higher absorption soluble co-precipitates (Muller et al., 1970). Insoluble and dispersed milk protein co-precipitates have also been considered as suitable ingredients for the fortification of breakfast cereals (Muller et al., 1970). Milk protein co-precipitates prepared by calcium

(0.5–0.8%) appeared to be the most soluble, and 1.0–1.5% calcium the least soluble (Kosaric and Ng, 1983).

An emulsion is a system in which the droplets of a liquid are dispersed in another liquid; it can be either oil in water or water in oil (Dickinson, 1988). Milk protein co-precipitates were found to improve the emulsifying and water binding capacity of the meat when 20% of the meat protein was replaced by the co-precipitates. When higher quantities of the co-precipitates were employed, both emulsion stability and water binding capacity of the meat decreased (Beuchat et al., 1975; Thomas et al, 1974).

Foams are dispersions of gas bubbles (air) in a continuous liquid or semi solid phase (Cheftel et al., 1985). Foaming capacity of milk protein co-precipitates reached a minimum at pH 4.5–5.0, then increased either in acidic or alkaline conditions, as a result of increased protein solubility (De Witt, 1989). Foaming stability for milk protein co-precipitates decreased by increasing protein concentration due to stronger protein interaction at high concentration (Kosaric and Ng, 1983). Milk protein co-precipitates exhibited better foaming properties when compared with other proteins, except soybean proteins (Kosaric and Ng, 1983).

An important property of protein gels is their water holding capacity (WHC) (Stanley and Yada, 1992). WHC is the weight of water held by 100 grams of a protein gel (Kinsella, 1984). Hermansoon (1986) defined it as a physical property, and is the ability of a gel to prevent water from being released from the three dimensional structure of the gel. WHC is affected by both calcium content and temperature during preparation. WHC of milk protein co-precipitates was measured by a method of Thomas et al. (1974). Protein gel may be defined as three dimensional matrices or network in which polymer-

polymer, and polymer-solvent interaction occurs in an ordered manner resulting in the immobilization of large amount of water by a small proportion of protein (Hermansson, 1979).

2.2.2 Plant Protein Co-precipitates

Seeds are potentially a source of low cost, edible vegetable proteins for supplementing dietary proteins shortage (Mattil, 1971). Recently many studies have been reported on the use of plant proteins to prepare new nutritionally balanced food, equal in nutritive value to protein diet from animal sources (Moharram and Abu-Foul, 1992; Abo-Foul et al., 1995).

2.2.2.1 Preparation of Plant Protein Co-precipitates

Hagan et al. (1986) prepared plant protein co-precipitates from soybean and peanut by mixing (1:1W/W) in water with a ratio 1:18 and stirring for 1h, followed by precipitation of curd by adjusting the pH to 4.5 with 1N HCl. The washed curd was finally adjusted to pH 7.0 with 1N NaOH and spray dried. Preparation of plant protein co-precipitates have been repeated from various combinations of processed cotton seed, soybean and peanut flour (1:1W/W), followed by acidification of the protein extract to pH 2.5, adjustment of the mixture to pH 5.0, and neutralization and lyophilization (Beradi and Cherry, 1979).

2.2.2.2 Properties of Plant Protein Co-precipitates

2.2.2.2.1 Composition of Plant Protein Co–precipitates

The advantage in using flour blends and co-precipitated protein isolates from two or more oilseed flours for human consumption has been recognized (Dendy et al., 1975; Tsen, 1976), Protein co-precipitates of plant source blends have shown higher nutritional value, and superior functional properties, lower level of anti-nutritional factors, higher in vitro protein digestibility and higher composition of essential amino acids, than those of the individual protein isolates (Youssef et al., 1995). Table 2.1 shows the proximate composition, functional properties and nutritional value of plant protein co-precipitates. Quintela et al. (1993) reported that the preparation of the protein co-precipitates resulted in the removal of most of the natural anti-nutritional factors in plant protein sources.

2.2.2.2.2 Functional Properties of Plant Protein Co-precipitates

Extrusion processing has been employed extensively for the production of texturized plant proteins for use as meat extenders, Twin screw texturization of a co-precipitated soy bean and peanut proteins resulted in the formation of a highly moist, less structurally rigid and moderately expanded product compared to individually textured soybean and peanut protein concentrates (Hagan et al., 1986).

Table 2.1: Proximate Composition, Functional Properties and Nutritional Value of Bean-Chickpea-Sesame Protein Co-precipitates.

Properties	Protein Co-precipitites
Proximate composition	
Moisture (%)	5.76
Crude protein (%) (Total N. X 6.25)	91.2
Ether extract (%)	0 .51
Ash (%)	4.22
Carbohydrate (%)	4.02
Functional properties	
Water absorption (%)	232
Fat absorption (%)	74.0
Emulsifying capacity (ml oil/100g flour)	79.0
Foaming capacity (%)	220
Nutritional value	
Tannis (mg/g)	0 .81
Phytic acid (mg/g)	7.69
Trypsin inhibitor (units/mg)	4.01
Chymotrypsin inhibitor (units/mg)	2.6
In vitro protein digestibility (%)	89.1
Amino acids (g/16 g N)	
Isoleucine	4.29
Leucine	8.35
Lysine	5.11
Methionine	1.62
Cysteine	0.58
Phenylalanine	4.73
Tyrosine	2.54
Threonine	3.11
Valine	4.58
Arginine	8.91
Histidine	2.81
Aspartic acid	9.46
Glutamic acid	15.3
Glycine	5.29
Serine	4.03

Source: Youssef et al., (1995).

2.2.3 Milk-Plant Protein Co-precipitates

A soy cheese-whey protein co-precipitate was prepared by Loewenstein and Paulraj (1972) from a concentrated mixture of defatted soy flour and cottage cheese whey. Plant-whey protein co-precipitates prepared by acid-heat processing have also seen reported (Morr, 1978). Table 2.2 shows the essential amino composition of protein isolate, concentrates and co-precipitates from rapeseed proteins and cheese whey protein.

2.2.3.1 Preparation of Milk-Plant Protein Co-precipitates

2.2.3.1.1 Preparation of Cheese Whey-Soy Protein Co-precipitates

Preparation of a protein co-precipitate from soy flour and cheese whey protein was done by the following procedure; defatted soy flour was dispersed in cottage cheese whey, the proteins was precipitated by heating the mixture to 98°C for 30 minutes, adjusting to pH 4.7, followed by neutralization and lyophilization (Loewnstein and Paulraj, 1972).

2.2.3.1.2 Preparation of Casein-Wheat Germ Protein Co-precipitate

Fayed (1987) prepared protein co-precipitates from wheat germ protein solution (3.5% protein, pH 9) and skim milk (pH 6.6) with a volume ratio of 30:70, respectively. The pH of the mixture after blending was 6.7-6.8. The pH of the mixture was raised to 9 by addition of 2M NaOH with stirring. Centrifugation (3000Xg/10 min) gave no protein. However, protein co-precipitate was obtained by adjusting the pH to 4.6 using HCl (2N).

Table 2.2: Essential Amino Acid Composition for Protein Isolates, Concentrates and Co-precipitates from Rapeseed Proteins and Cheese Whey Protein.

Essential Amino Acids as (%of Total)							
Products	Lys	Thre	Met	Val	Ileu	Leu	Tyr Phe
Rapeseed flour (RF)	15.1	11.3	8.9	14.5	12.2	20.8	17.2
Rapeseed isolate (RI)	10.4	123.2	6.0	14.8	12.4	20.7	22.5
Rapeseed isolate whey co-precipitate (RIW)	17.0	12.4	8.4	12.3	10.7	21.7	17.5
Rapeseed isolate whey co-precipitate (theoretical) (RIW)	14.0	12.3	7.3	13.4	11.4	21.2	19.6
Rapeseed concentrate (RC)	14.9	13.1	8.6	13.6	9.0	20.2	20.9
Rapeseed concentrate whey co-precipitate (RCW)	17.1	12.5	7.9	12.8	8.9	22.8	18.2
Whey (W)	19.6	10.7	9.2	11.0	9.7	21.9	14.9

Source: Thompson (1977).

2.2.3.2 Functional Properties of Milk-Plant Protein Co-precipitates

Milk-plant protein co-precipitates from whey and bean protein have been reported to show improved functional properties compared to the bean and whey proteins individually (Kebary, 1993). Tables 2.3 and 2.4 show some functional properties of milk–plant protein co-precipitates.

Protein solubility is important for the application of proteins in beverages, infant formula, texturized meats, and sauce, and as an index of protein changes during food processing (Kebary, 1993). Protein solubility of whey-bean protein co-precipitates reached the minimum around pH 4.5–5, the region of isoelectric points of the proteins (De Witt, 1989). Milk-plant protein co-precipitates carry negative and positive changes above and below the isoelectric point respectively, and water molecules can interact with these charges to enhance the solubility (Kebary, 1993). In general proteins showed higher solubility at alkaline pH (7-10) than at acid pH (2-5), while the minimum solubility is observed at around pH 4.5 (Fayed, 1997).

The formation and stabilization of emulsions is critical for many applications such as chopped, cakes, salad dressings, coffee whiteners, and comminuted meats. The emulsion capacity of milk-plant co-precipitates was reported to be a minimum around pH 4.5–5.0, then increased in both sides of this pH range, emulsion properties were also affected by NaCl (Kebary, 1993).

The foaming capacity of a milk–plant protein co-precipitate increased by increasing the NaCl concentration up to 0.4M and then decreased (Kebary, 1993). The plant protein co-precipitate showed the highest foaming stability at pH 10.0 with decrease in foaming stability with decreasing to pH 4.0 (Kebary, 1993).

Table 2.3: Functional Properties for Protein Isolates, Concentrates and Co-precipitates from Rapeseed Proteins and Cheese Whey Protein.

Products	Color Y_{CIE}	pH of 10% Dispersion	Nitrogen Solubility%		Water Absorption %	Fat Absorption %	Emulsifying Capacity (mlOil/20ml sample)	Whipping Capacity (%)
			H_2O %	0.2% NaOH				
RF	75.8	5.5	48.8	100	174.5	146.2	34.5	73.7
RI	29.8	7.3	9.2	100	273.0	105.5	13.0	37.0
RIW	32.8	7.3	8.8	100	362.3	121.3	20.5	25.4
RC	54.7	7.3	6.4	100	405.6	198.0	13.5	13.2
RCW	58.8	7.3	10.7	100	239.9	127.0	17.5	35.6
WC	82.9	7.2	8.0	100	255.9	153.9	22.0	32.0

Source: Thompson (1977).
For abbreviations, see Table 2.2.

Table 2.4: Water Absorption, Oil Absorption and Gelation Ability of Whey Proteins, Bean Proteins and Whey-Bean Protein Co-precipitates.

Functional Properties	Bean Protein	Whey Protein Bean Protein Co-precipitates	Whey Protein
Water absorption (g H_2O/100 g sample)	275.98	190.69	147.78
Oil absorption (ml oil/100 g sample)	107.6	99.6	82.6
Protein concentration to form gel (% protein)	7.0	4.0	6.5

Source: Kebary (1993).

A protein co-precipitate from whey and bean proteins showed improved gelation properties compared with whey and bean proteins individually (Catsimpoolas and Meyer, 1970). The whey-bean protein co-precipitates also showed improved water and oil absorption (Kebary, 1993).

2.2.4 Other Protein Co-precipitates

Whey proteins have been co-precipitated with other proteins such as blood and egg proteins. Maximum yield was obtained when acid in conjunction with low levels of $CaCl_2$ was used (Mathure and Shahani, 1977; Hill et al., 1982). Whey-blood protein co-precipitates was found to be a poor protein source because the heat treatment required for its preparations destabilized the whey proteins causing a decrease in the protein efficiency ratio and the biological value of the blood proteins (Young, 1980). Chemical precipitation agents have been used to prepare egg protein–whey protein and blood protein–whey protein co-precipitates (Schmidt and Illingworth, 1978). Whey-egg protein co-precipitate showed a higher WHC than proteins from whey powder and egg powder individually (Kosaric and Ng, 1983).

2.3 Proteins from Soybean and Whey

Soybean proteins consist primarily of 2S, 7S, 11S and 15S fractions (Yamuchi et al., 1991). Table 2.5 shows the components of soybean proteins. The 11S component (glycinin), has a molecular weight (MW) of about 360 KDa and is composed of acidic (A_1-A_4, 34-45 KDa) and basic (B_1-B_4, 18-22 KDa) polypeptide chains linked by a disulfide bond (A_n-S_S $_{Bn}$) (Kitamura et al., 1976; Staswikj et al., 1984). Glycinin is a

single protein (Liu, 1997); it consists of six subunits, which each glycinin subunits consists of two polypeptide components, one with acidic and another one with basic isoelectric point (Zarins and Marshall, 1990; Petruccelli and Anon, 1995).

Conglycinin, a 7S globulin (MW about 140-170 KDa) exists in at least seven forms (B_0-B_6) as a result of various combinanitions ofα, α', and β subunits (MW 57-68 KDa, 57-72 KDa and 42-52 KDa, respectively) (Thanh and Shibasaki, 1978; Yamauchi et al., 1981). The 2S fraction contains several trypsin inhibitors, cytochrome C, and undefined proteins, raw soybean contains 14% Kuntiz inhibitor and 0.6% Bowman-Brik inhibitor (Vaidehi and Kadam, 1989). The Kunitz inhibitor has a MW of 20 KDa with two disulfide bridges (Koide and Ikenaka, 1973). The Bowman-Brik trypsin inhibitor has low MW (8 KDa) 7 disulfide bridges (Odani et al., 1972), so that it possesses a high thermal stability (Dipietro and Liener, 1989).

Soybean agglutinin is a tetramer (MW 120 KDa) composed of identical subunits (MW 30 KDa) lacking disulfide bridge having two saccharide binding sites and an IEP of 5.8 (Lotan et al., 1974). Some soybean proteins, as the trypsin inhibitors, contribute to the nutritional quality of soybean by virtue of their relatively high cysteine content (Tan-wilson and Wilson, 1986). The functional properties of soy proteins are affected by size, amino acid composition, net charge, sequence of amino acid, method of extraction, isolation of pH, temperature and ionic strength (Zayaz, 1997).

Whey is the soluble fraction of milk that is separated from casein curd during cheese manufacturing; the main proteins in whey are β-Lactoglobulin (β-lg), α-lactalbumin (α-la), proteose-peptone, immunoglobulin, and bovine serum albumin (BSA) (Aguilera, 1995). The distribution of the whey proteins in whey is shown in Table 2.6.

Table 2.5: Components of Water Extractable Soybean Proteins.

Fraction	% of Total	Components	Molecular Weight Da
2S	22	Trypsin inhibitor	8000-21500
		Cytochrome C	12000
7S	37	Hemagglutinin	110000
		Lipoxygenase	1020001
		β-Amylase	61700
		7-S Globulins	140000-210000[a]
11S	31	11-S Globulin	350000-360000[b]
15S	11		600000

Source: (a): (Wolf (1970); Thanh and Shibasaki (1978); Yamauchi et al. (1981)); (b): (Wolf (1970); Kitamura et al. (1976); Staswikj et al. (1984));

β-Lg is a globular protein having a molecular weight of 18,000 Da (Swaisgood, 1982; Papiz et al., 1986). α-La is a compact globular protein having a molecular weight of 14,000 Da (Swaisgood, 1982). The major whey proteins that comprise 20% of milk proteins are lost in whey during the manufacture of cheese (Kebary, 1993). The secondary structure of β-lactoglobulin, contains 33% α-helix, 33% β-sheet structure at neutral pH. It exists as a dimer, but in other conditions, it dissociate to the monomer. which consists of five cysteine residues (Pearce, 1989). The secondary structure of α-La, shows of 26% α-helix, 14% β-structure and 60% unordered structure (Bottomley et al., 1990).

Table 2.6: Distribution and Properties of Proteins Whey.

Proteins	g/l	Molecular Weight (Da)	Isoelectrical Point pH
β-Lactoglobulin	2.0-4.0	18, 000-18,362	5.35-5.41
α-Lactalbumin	1.0-1.7	14,000-14,174	4.2-4.5
Bovine Serum Albumin	0.1-0.4	66, 500-69,000	5.13
Immunoglobulin	0.6-1.0	150,000-1000000	5.5-8.3
Proteose Peptone	0.6-1.8	4,100-40,800	3.3-3.7

Source: (Mulvihill and Kinsella (1987); (Swaisgood (1982); Papiz et al. (1986)).

2.4 Gelation and Rheological Properties of Whey Proteins and Soybean Proteins

Heat denaturation and gel formation of soy proteins have been extensively studied. Heat denaturation is often a prerequisite for gel formation. Glycinin has a denaturation temperature of 90°C at neutral pH and an ionic strength of 0.25 M; reducing the ionic strength lowers the denaturation temperature (Hermansson, 1986; Damodaran, 1988).The gel characteristics of pure glycinin are affected by ionic strength (Utsumi and Kinsella, 1985; Van Kleef, 1986), heating temperature (Nagano et al., 1994a) and pH during heating (Van Kleef, 1986; Nagano et al., 1994b). Heat treatment induces denaturation and aggregation of soy protein molecules; at high protein concentration greater than 7%W/W in soy proteins, the aggregates formed produce a self-supporting gel (Hermansson, 1978; Van Kleef, 1986).

The gelation process of whey proteins is considered as a series of reactions (Foegeding et al., 1995). Whey protein has compact three-dimensional structures of folded amino acid chains maintained by non-covalent interactions and covalent disulfide bonds. Heat induced protein gelation begins with conformational changes resulting in partial unfolding of proteins, exposing interior reactive regions and sulphydryl groups of the proteins (Qi et al., 1997; Belloque and Smith, 1998). The gelation of whey proteins is influenced by protein concentration, temperature, heating methods (heating/cooling rate), environmental conditions (pH, ionic strength) and interaction with order food components (salt, sugar, glucose) (Damodaran, 1989). Heat induced gelation of whey powder involves an initial denaturation/unfolding step followed by aggregation into protein particles adhering as a network (Mulvihill and Kinsella, 1987). Whey protein

forms different network structures depending on pH; aggregated particulate networks are formed at intermediate pH (4-6), and fine stranded networks at high or low pH away from the isoelectric point (Langton and Hermansson, 1992). The aggregated gels show higher elastic properties than fine-stranded gels (Stading and Hermansson, 1990).

Chronakis and Kasapis (1993) studied the rheological properties of mixed soy-whey gels using a fixed amount of whey protein (10/W/W) with a range of soy protein concentration (6-16%W/W); the stability of the mixed protein dispersion was improved by increasing the pH and the temperature. The viscoelastic properties include the storage modulus (G') and loss modulus (G"), (G') a measure of the energy stored and is related to gel elasticity; while (G") is a measure of energy dissipated per cycle and is related to gel viscosity (Hamann et al., 1990).

2.5 Protein Interaction During Formation of Protein Co-precipitates

2.5.1 Protein Interaction Without Denaturation for Protein Co-precipitates

Native plant storage proteins possess regular quaternary structures which seem to differ little between plant species (Plietz et al., 1984); generally, there seem to be two types of protein having sedimentation coefficients in the region of 7S and 11S. The 7S protein (soybean conglycinin) is composed of six similar sized subunits (Kinsella et al., 1985) held together mainly by hydrophobic bonds, the 7S can also dimerize when the ionic strength is increased (Plietz et al., 1984). The 11S molecules is composed from six subunits, each of which subunits contains two disulfide-linked protein molecules (A and B) (Catsimpoolas et al., 1971), which form an octahedral complex (Plietz et al., 1983; Plietz et al., 1986).

Raising or lowering the pH can dissociate the subunits, but in the absence of reducing agents the A and B proteins remain linked by their disulfide bonds (Gueguen et al., 1988). These interactions are summarized in Figure 2.1.

In contrast to the plant proteins, the different types of caseins, the β, α and K-casein, all show different, but much less regular behavior patterns when they are dissolved in aqueous solution. The simplest, as casein, polymerizes to a small extent, to form small oligomers, but does not give substantially larger particles. β- Casein forms multimers containing up to about 60 protein molecules linked together by hydrophobic bonds (Andrews et al., 1979; Kajiwara et al., 1988). In β-casein aggregates, no covalent bonds are formed; in particular, the proteins lacks cystine and therefore cannot make intermolecular disulfides (Pepper and Farrell, 1982). These complexes may exist naturally and be partly responsible for the structure of the casein micelle.

β-Lactoglobulin exists mainly as a dimer; the nature of the interaction between the two parts of the dimer has been described (Papiz et al., 1986), Hydrophobic effects are important, as are other more specific interactions between amino acids. The dimer may be dissociated by lowering the pH to below 3.5 or by raising the temperature to above 40°C (Townend et al., 1960). In addition, the protein can form octameric aggregates at pH values close to its isoelecric point (pH 5.1) (Pessen et al., 1985).

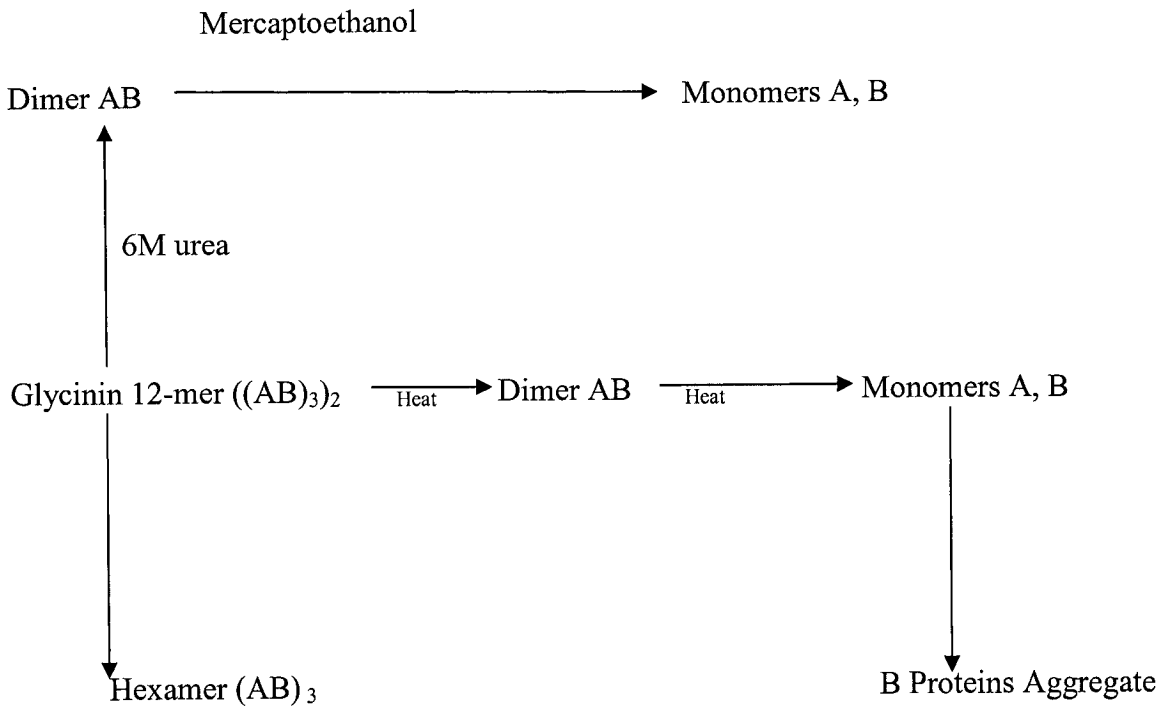


Figure 2.1: Summary of the major interactions involved during heating of glycinin (Source: Dalgleish and Hunt, 1995).

2.5.2 Interaction of Protein During Heating for Protein Co-precipitation

In general when proteins are heated, they undergo structural changes, most of which are irreversible. Proteins such as α-lactalbumin or β-lactoglobulin show well defined thermal transitions in the range between 60 and 80°C, depending on the concentration of the protein and the composition of the buffer in which they are dissolved (Harwalkar and Ma, 1992). As denaturation proceeds, at least some secondary structure is lost, and there may be a randomization of disulfide interactions (Jang and Swaisgood, 1990). Differences exist between proteins as to the reversibility of the thermal transition; once β-lactoglobulin has been thermally denatured, it remains so, but there seems to be some reversibility to the denaturation of α-lactalbumin (Paulsson and Visser, 1992).

After the proteins have seen denatured by heat, it is possible for them to react further. At temperatures in excess of 70°C, the milk serum proteins can form gels by interactions with, the K-casein, and also possibly with the α-casein (Jang and Swaisgood, 1990). Heating also has affects plant storage proteins. One effect of heating is to dissociate both β-conglycinin and glycinin into their subunits (German et al., 1982). In glycinin, the AB dimers forming the original hexameric complex separate as a result of breaking of the disulfide bonds (Utsumi et al., 1984). The separated proteins then begin to aggregate, which is accentuated if β-conglycinin is present; the B proteins of glycinin aggregate with the B protein of the β-conglycinin trimer (Utsumi et al., 1984). The A proteins are much more soluble and participate in complex formation to a much lesser extent (Yamagishi et al., 1980).

2.5.3 Protein Gelation of Protein Co-precipitates

Protein aggregation often leads the formation of a gel; the ability of proteins to form a gel has seen exploited for many food applications (Ziegler and Foegeding, 1990). Gels are structural networks formed from a limited number of specific protein-protein interactions, immersed in a liquid medium, which maintains its shape under gravity and has mechanical strength while retaining many characteristics of a fluid (Ziegler and Acton, 1984). The physical attributes of protein gels are determined by the type and number of protein-protein interactions. These interactions are sensitive to variables such as solvent characteristics (pH, ionic strength, etc.), and heat (Ziegler and Foegeding, 1990; Ziegler and Acton, 1984).

2.5.3.1 Mechanism of Gelation for Protein Co-precipitates

The mechanism of heat induced gelation of globular proteins is not understood fully, the most commonly accepted scheme for gelation involves two steps (Ferry, 1948): a denaturation step during which the native protein conformation is altered either by heat or chemically followed by an aggregation step during which the denatured protein molecules become oriented to produce a gel network. Some degree of protein denaturation is a prerequisite for gelation (Ferry, 1948; Clark and Lee-Tuffnell, 1986) to expose parts of the protein molecule that facilitate intermolecular interactions. The cysteine-120 group of β-lactoglobulin is exposed readily during heating (Kella and Kinsella, 1988); this facilitates -SH/S-S interchange. The surface hydrophobicity of soy protein preparations increases during heating because the glycinin protein dissociates into subunits (Koshiyama et al., 1981). There is, on average, more than two of these "active

sites" exposed during denaturation for a three-dimensional gel network to develop (Bernal and Jelen, 1985; Mulvihill and Kinsella, 1987).

During gelation the extent of aggregation is determined by the balance of attractive interactions and repulsive forces between the denatured protein molecules (Harwalkar and Kalab, 1985); the extent of these interactions depends on pH and ionic strength (Stanley and Yada, 1992; Koning and Visser, 1992), protein concentration, amino acid composition and molecular weight (Shimada and Matsushita, 1980; Kohnhorst and Mangino, 1985), and heating/cooling rates (Foegeding et al., 1986). The presence of other components can also influence the balance between attractive and repulsive forces. Calcium can form intermolecular protein Ca-protein bridges (Lupano et al., 1992). Urea can disrupt hydrophobic interactions and hydrogen bonding (Xiong and Kinsella, 1990), and ethanol can increase electrostatic interaction by reducing the dielectric constant of the solvent (Zirbel and Kinsella, 1988).

2.5.3.2 Protein-Protein Interactions in Gelation of Protein Co-precipitates

A. Hydrophobic Interactions and Hydrogen Bonds

When globular proteins are dissociated or unfolded by heating, they expose previously buried hydrophobic groups. Highly hydrophobic proteins such as hemoglobulin, catalyase and egg albumin tend to form opaque coagula when heated, as opposed to gels; it appears that the large numbers of hydrophobic interactions produce dense networks which bring about separation of the charged amino acid residues and this may suppress electrostatic repulsion between the molecules (Shimada and Matsushita, 1980). Unfolded proteins may have regions with the potential to form intermolecular

hydrogen bonds (Brandenberg et al., 1992). Heating breaks the hydrogen bonds, as evidenced by the loss of α-helices during heat gelation of bovine serum albumin (Clark et al., 1981). Hydrogen bonding has been shown to be important in the heat-induced gelation of soy β-conglycinin globulin at pH 8 (Babajimopoulos et al., 1983; Utsumi and Kinsella, 1985). Hydrogen bonding is thought to be important to gel stabilization only at acid pH values, where the extent of other protein-protein interaction is limited (Shimada and Cheftel, 1988).

B. Electrostatic Interactions.

Salts strongly affect the strength, deformability, and appearance of protein gels (Lupano et al., 1992; Kuhn and Foegeding, 1991). Calcium ions influence protein-protein interactions by shielding electrostatic repulsion and also by forming protein-Ca-protein bridges (Lupano et al., 1992). The presence of calcium was found to decrease aggregation temperature and increase the aggregation rate of whey protein isolate (Xiong, 1992). The effect of NaCl on gel strength essentially parallels that of $CaCl_2$, although it is required in greater concentration to produce the equivalent effect. Despite the parallel effects of Ca and Na on protein gel strength, the gels that are formed differ in appearance and in deformability (Kuhn and Foegeding, 1991).

Intermolecular disulfide bridges are important with respect to the functional properties of food proteins; for example, they contribute towards the viscoelastic behavior of β-lactoglobulin films absorbed at the oil-water interface (Dickinson et al., 1990), and are responsible for covalent binding between α-lactalbumin and β-lactoglobulin, and K-casein when milk is heated (Jang and Swaisgood, 1990). There seems to be some disagreement as to the structural importance of intermolecular disulfide

bridges in heat induced protein gels. It may be that, rather than forming network structure directly, S-S bonds promote gelation by increasing polypeptide chain length (Damodaran, 1989), or perhaps the precise distribution and accessibility of -SH and S-S groups are important in determining the structural and functional significance of disulfide bridges. The formation and function of disulfide bridges are dependent on other variables such as pH and ionic strength, which in turn determine the extent of other protein-protein interactions (Dalgleish and Hunt, 1995).

2.5.3.3 Factors Affecting Gelation of Protein Co-precipitates

2.5.3.3.1 Effect of pH and Ionic Strength

Protein gelation depends on the balance of attractive and repulsive forces and on net charges; as a result, pH and ionic strength influence the extent of protein-protein interactions and therefore must be considered. When far removed from the isoelectric point, electrostatic repulsion inhibits intermolecular interactions and gel structure is poor. The macroscopic structure of the protein gels varied with pH close to the IEP; at pH values further from the IEP, electrostatic repulsion increased, intermolecular interaction decreased, and gels became more translucent (Egelandsdal, 1980). Proteins are most heat sensitive around their isoelectric points (De Witt, 1981); therefore, they unfold more readily to expose reactive sites for intermolecular crosslinking. Increased ion concentration in protein solutions, shields electrostatic repulsion and increases the potential for intermolecular associations (Kuhn and Foegeding, 1991). Controlling ionic strength and/or pH enables gel strength to be maximized. Gel properties cannot be ascribed to one type of protein-protein interaction alone, since a number of interaction

mechanisms must be considered; the solvent conditions (pH, ionic strength, etc.) help to determine which interactions will prevail.

2.5.3.3.2 Effect of Other Constituents

Ethanol decrease the dielectric constant and therefore enhances electrostatic attractions between proteins (Creighton, 1983). The hardness of gels increased upon the addition of ethanol (Zirbel and Kinsella., 1988). Urea destabilizes or perturbs hydrophobic interactions and hydrogen bonds in proteins (Lapanje, 1978); therefore, in the presence of urea, the proteins dissociate, exposing free thiol groups, which facilitate intermolecular SH/SS interchange with concomitant network formation and gelation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Commercial defatted soybean flour (50 % protein, 1.2 % fat, 3.5 % fiber and 8 % moisture) was obtained from Daminco Inc. (Dorval, Quebec, Canada) and stored in containers at 4°C. Commercial samples of whey powder concentrate (35 % protein, 4 % fat, 5 % moisture and 7 % ash) was obtained from Agropur Cooperative (St. Principe, Granby, Quebec, Canada) and stored in containers at 4°C.

3.2 Preparation of Whey Protein-Soybean Protein Co-precipitates

3.2.1 Preparation of Co-precipitates Using Mixed Soybean Flour/Whey Powder (MP)

3.2.1.1 Sodium Hydroxide Extraction/Cooling Co-precipitation (MP:NaOH/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Davidson et al. (1979) and Alli and Baker (1980) with modifications.

Extraction: Defatted soybean (15g) and whey powder (7.5g) were mixed with dilute NaOH (2N, 128ml, pH 11). The mixture was adjusted to pH 11 and stirred (40°C/60 min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and the residue was discarded.

Co-precipitation: The extract was refrigerated (4°C/24h), the co-precipitate was recovered by centrifugation (10,000Xg/30 min) followed by lyophilization. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.1.2 Sodium Hydroxide and Papain Extraction/Cooling Co-precipitation (MP:NaOH-Papain/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Pallavicini and Trentin (1987) and Alli and Baker (1980), with modifications. *Extraction:* Defatted soybean (15g) and whey powder (7.5g) were mixed with dilute NaOH (2N, 128ml, pH 11). The mixture was adjusted to pH 11, stirred (40°C/60 min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and the residue was discarded. The extract was adjusted to pH 11 with NaOH (2N), pre-incubated (37°C/15 min), then papain (0.3ml, 10mg/ml) was added. The mixture was incubated (37°C/6h) in a water bath, then acidified with citric acid (0.2%) (W/V) to inactivate the enzyme.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.1. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.1.3 Sodium Hydroxide Extraction/IEP and Cooling Co-precipitation (MP:NaOH/IEP-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Johnson and Kikuchi (1988), Waggle et al. (1989) and Alli and Baker (1980), with some modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.1.1.

Co-precipitation: The extract was adjusted to pH 4.6 by dropwise addition of HCl (2N) with continuous stirring, then refrigerated (4°C/24h). The co-precipitates were recovered by centrifugation (10,000Xg/30 min) followed by lyophilization. Controls were prepared from whey powder alone (5% W/V) and soybean flour alone (10% W/V) using the same conditions and procedures.

3.2.1.4 Sodium Hydroxide Extraction/IEP, Heating and Cooling Co-precipitation (MP:NaOH/IEP-Heating-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Johnson and Kikuchi (1988), Waggle et al. (1989) and Alli and Baker (1980), with modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.1.1.

Co-precipitation: The extract was adjusted to pH 4.6 by dropwise addition of HCl (2N) with continuous stirring, heating (95°C/30 min), then refrigerated (4°C/24h). The co-precipitates were recovered by centrifugation (10,000Xg/30 min) followed by

lyophilization. Controls were prepared from whey powder alone (5% W/V) and soybean flour alone (10% W/V) using the same conditions and procedures.

3.2.1.5 Sodium Hydroxide and NaCl Extraction/Cooling Co-precipitation (MP:NaCl-NaOH/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Youssef et al. (1995) and Alli and Baker (1980), with modifications.

Extraction: Defatted soybean (15g) and whey powder (7.5g) were mixed with (15g) of NaCl and dilute NaOH (2N, 113ml, pH 11). The mixture was adjusted to pH 11, stirred (40°C/60 min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and the residue was discarded.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.1. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.1.6 Sodium Hydroxide and NaCl Extraction/IEP-Cooling Co-precipitation (MP:NaCl-NaOH/IEP-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Youssef et al. (1995) and Alli and Baker (1980), with modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.1.5.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.3. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2 Preparation of Co-precipitates Using Mixed Whey Extract/Soybean Extract (ME)

3.2.2.1 Preparation of Soybean Protein Extract

The procedure for protein extraction was based on previous work by Davidson et al. (1979), with modifications. Defatted soybean flour (15g) was mixed with dilute NaOH (2N, 135ml, pH 11), the slurry was stirred (40°C/60 min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and retained for use in Sections 3.2.2.3 and 3.2.2.4; the residue was discarded.

3.2.2.2 Preparation of Whey Protein Extract

The procedure for protein extraction was based on previous work by Davidson et al. (1979), with modifications. Whey powder (7.5g) was mixed with dilute NaOH (2N, 142ml, pH 11), the alkaline slurry was stirred (40°C/60min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and retained for use in Sections 3.2.2.3 and 3.2.2.4; the residue was discarded.

3.2.2.3 Sodium Hydroxide Extraction/Cooling Co-precipitation (ME:NaOH/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Davidson et al. (1979) and Alli and Baker (1980), with modifications.

Extraction: The protein extract from soybean flour Section (3.2.2.1) and from whey powder Section (3.2.2.2) were mixed, the mixture after blending was adjusted to pH 11 followed by stirring (40°C/60 min) in a water bath.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.1. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2.4 Sodium Hydroxide and Papain Extraction/Cooling Co-precipitation (ME:NaOH-Papain/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Pallavicini and Trentin (1987) and Alli and Baker (1980), with modifications.

Extraction: The protein extract from soybean flour Section (3.2.2.1) and from whey powder Section (3.2.2.2) were mixed, the mixture was adjusted to pH 11 followed by stirring (40°C/60 min) in a water bath. The mixture was pre-incubated (37°C/15 min), then papain solution (0.3ml, 10mg/ml) was added to the mixture, the mixture was incubated for (37°C/6h) in a water bath, and then acidified with citric acid (0.2% W/V) to inactivate the enzyme.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.1. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2.5 Sodium Hydroxide Extraction/IEP and Cooling Co-precipitation (ME:NaOH/IEP-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Youssef et al. (1995) and Alli and Baker (1980), with modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.2.3.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.3. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2.6 Sodium Hydroxide Extraction/IEP, Heating and Cooling Co-precipitation (ME:NaOH/IEP-Heating-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Kebary (1993) and Alli and Baker (1980), with modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.2.3.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.4. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2.7 Sodium Hydroxide and NaCl Extraction/Cooling Co-precipitation (ME:NaCl-NaOH/Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Youssef et al. (1995) and Alli and Baker (1980), with modifications.

Extraction: Defatted soybean flour (15g) and NaCl (15g) were mixed with dilute NaOH (2N, 120ml, pH 11), the mixture was stirred (40°C/60 min) in a water bath, then centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and the residue was discarded. Whey powder (7.5g) and NaCl (15g) were mixed with dilute NaOH (2N, 127ml, pH 11), the slurry was stirred (40°C/60 min) in a water bath, then mixture was centrifuged (10,000Xg/30 min). The extract was filtered through glass wool and the residue was discarded.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.1. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.2.2.8 Sodium Hydroxide and NaCl Extraction/IEP and Cooling Co-precipitation (ME:NaCl-NaOH/IEP-Cooling)

The procedure for protein co-precipitate preparation was based on previous work by Youssef et al. (1995) and Alli and Baker (1980), with modifications.

Extraction: The extraction procedure was similar to that described in Section 3.2.2.7.

Co-precipitation: The co-precipitation procedure was similar to that described in Section 3.2.1.3. Controls from whey powder alone (5% W/V) and soybean powder alone (10% W/V) were prepared using the same conditions and procedures.

3.3 Factors Affecting on Yield of Co-precipitate

3.3.1 Effect of Chelating Agents Na-HEXA and Ca-EDTA on Yield

3.3.1.1 Effect of Ca-EDTA and Na-HEXA on Yield of MP:NaOH/Cooling Co-precipitate

The effects of (a) Ca-EDTA (0, 0.5, 1.5, and 2.5 % W/V), (b) Na-HEXA (0, 0.5, 1.5, and 2.5 % W/V) and (c) mixture of Ca-EDTA and Na-HEXA (0:0, 0.5:0.5, 1.5:1.5, and 2.5:2.5 % W/V) on the yield of protein co-precipitate obtained by the extraction and co-precipitation procedure described in Section 3.2.1.1, were investigated. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.1.2 Effect of Ca-EDTA and Na-HEXA on Yield of MP:NaOH Extraction/IEP-Cooling Co-precipitate

The effects of (a) Ca-EDTA (1.5 % W/V), (b) Na-HEXA (2.5 % W/V) and (c) mixture of Ca-EDTA and Na-HEXA (1.5:2.5 % W/V) on the yield of protein co-precipitate obtained by the extraction and co-precipitation procedure described in Section 3.2.1.3, were investigated. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.1.3 Effect of Ca-EDTA and Na-HEXA on Yield of ME:NaOH/IEP-Cooling Co-precipitate

The effects of (a) Ca-EDTA (1.5 % W/V), (b) Na-HEXA (2.5 % W/V) and (c) mixture of Ca-EDTA and Na-HEXA (1.5:2.5 % W/V) on the yield of protein co-precipitate obtained by the extraction and co-precipitation procedure described in Section 3.2.2.5, were investigated. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.1.4 Effect of Ca-EDTA and Na-HEXA on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate

The effects of (a) Ca-EDTA (1.5 % W/V), (b) Na-HEXA (2.5 % W/V) and (c) mixture of Ca-EDTA and Na-HEXA (1.5:2.5 % W/V) on the yield of protein co-precipitate obtained by the extraction and co-precipitation procedure described in Section 3.2.1.4, were investigated. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.1.5 Effect Ca-EDTA and Na-HEXA on Yield of ME:NaOH/IEP-Heating-Cooling Co-precipitate

The effects of (a) Ca-EDTA (1.5 % W/V), (b) Na-HEXA (2.5 % W/V) and (c) mixture of Ca-EDTA and Na-HEXA (1.5:2.5 % W/V) on the yield of protein co-precipitate obtained by the extraction and co-precipitation procedure described in Section

3.2.2.6, was investigated. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.2 Effect of pH of Precipitation on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate

Extraction: The extraction procedure was similar to that described in Section 3.2.1.1.

Co-precipitation: The extracts were adjusted to pH 3.5, 4, 4.5, 5 and 6 by dropwise addition of HCl acid (2N) with continuous stirring and heating (95°C/30 min). The extracts were refrigerated (4°C/24h), the protein co-precipitates was recovered by centrifugation at (10,000Xg/30 min) and then lyophilized. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.3.3 Effect of Temperature of Precipitation on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate

Extraction: The extraction procedure was similar to that described in Section 3.2.1.1.

Co-precipitation: The extracts were adjusted to pH 4.5 by dropwise addition of HCl acid (2N), then heated (60, 70, 80, 90, and 100°C) for 30 min with continuous stirring. The extracts to stand in refrigerated (4°C/24h), the protein co-precipitates was recovered by centrifugation at (10,000Xg/30 min) and then lyophilized. The protein yield was calculated on the basis of the protein content of the co-precipitates.

3.4 Protein Content and Yield

Protein content was determined by the micro-Kjeldahl method (AOAC, 1980). %Nitrogen content was converted to %protein content by using factors of 5.95 for protein co-precipitates, 5.52 for soybean flour and 6.38 for whey powder. Nitrogen recoveries were obtained from the nitrogen content of the defatted soybean flour and whey powder and the sample weight. Protein yield was calculated on weight basis and on the basis of protein contents. All determinations for the yields were performed in triplicate, and for protein contents in duplicate and the standard error of the mean (SEM) was calculated.

3.5 Ash Content

Ash content was determined by ashing in a muffle furnace (570°C/7h) according to AOAC (1980) method. All determinations were performed in triplicate.

3.6 Polyacrylamide Gel Electrophoresis (Native PAGE)

Polyacrylamide gel electrophoresis (PAGE) was performed under native conditions according to the method of Davis (1964), using a mini-protean II electrophoresis cell unit (Bio-Rad, Hercules, CA). Polyacrylamide gradient gels (4%-20%) were used. Sample solutions (15μl), prepared from of freeze-dried protein co-precipitates (30mg) and supernatants (35mg) dissolved in sample buffer (1ml) (1.5M Tris HCl pH 8.8, 2% glycerol and 0.1% bromophenol blue), were applied to each sample well. Electrophoretic migration was performed for 2-3h at constant current (10mA/gel) using Tris-glycine buffer (10% of pH 8.3). Gels were stained with Coomassie Brilliant Blue R-

250 (0.1% W/V) in water (70%), methanol, (20%), acetic acid (10%) and destained with the same solvent system (but without dye).

3.7 SDS-PAGE

SDS-PAGE electrophoresis was carried out on gradient gels (4-20%) using the technique described by Laemmli (1970). The freeze-dried protein samples prepared from protein co-precipitates (3mg) and supernatants (5mg) dissolved in sample buffer (1ml) (10% SDS, 0.5M β-mercaptoethanol, 0.5M Tris HCl pH 6.8, 2% glycerol and 0.1% bromophenol blue), were heated (95°C, 5-10 min). Electrophoresis was performed at constant current (15 mA/gel) for 1.5-2h. The protein sub-units bands were stained with Coomassie Brilliant Blue R-250 (0.1%) in water (70%), methanol (20%), acetic acid (10%), and destained in the same solvent system (but without dye). A mixture of the marker proteins (Bio-Rad Hercules, CA) treated with SDS was subjected to the same procedure as described above.

3.8 Light Compound Microscopy

The following samples were subjected to Light Compound Microscopy (LCM): MP:NaOH/IEP-Cooling, MP:NaOH/IEP-Heating-Cooling, MP:NaCl-NaOH/IEP-Cooling, ME:NaOH/IEP-Cooling, ME:NaOH/IEP-Heating-Cooling and ME:NaCl-NaOH/IEP-Cooling for protein co-precipitates, whey protein precipitate:NaOH/IEP-Cooling, whey protein precipitate:NaCl-NaOH/IEP-Cooling, soybean protein precipitate:NaOH/IEP-Cooling and soybean protein precipitate:NaCl-NaOH/IEP-Cooling. A suspension of the protein was fixed on microscopy slide and examined under

a 1000X objective lens; the samples were photographed with a bright field photomicroscope and using a Fuji film camera. All determinations were performed in two test views per test.

3.9 Rheological and Gelation Properties of Protein Co-precipitates

3.9.1 Preparation of Gels

Protein gels were prepared from the following samples: MP:NaOH/IEP-Cooling, MP:NaOH/IEP-Heating-Cooling, ME:NaOH/IEP-Cooling and ME:NaOH/IEP-Heating-Cooling for protein co-precipitates, whey protein precipitate:NaOH/IEP-Cooling, whey protein precipitate:NaOH/IEP-Heating-Cooling, soybean protein precipitate:NaOH/IEP-Cooling and soybean protein precipitate:NaOH/IEP-Heating-Cooling. Aqueous dispersions (8%, 12% and 16%) of the samples above were prepared in distilled water in beakers and mixed to obtain a uniform suspensions; the was adjusted to pH 8 by dropwise addition of NaOH (0.1N). The beakers were covered with aluminum foil to prevent moisture loss, and were heated in a water bath (95°C/90 min). Gels were kept at (4°C/24h) before removal the gel from the beakers for gelation studies.

3.9.2 Gel Strength Measurements

Gel strengths of gels prepared in Section 3.9.1 were determined according to a modification of the procedure as described by Mulvihill and Kinsella (1988). Gels were uniaxially compressed to 50% deformation using the Universal Instron-Testing Machine (Instron Canada, Model 4502, Series IX, Burlington, Ontario). The gel strength was

calculated as the force required breaking the gels (load at yield); all measurements were done in duplicate.

3.9.3 Water Holding Capacity (WHC)

WHC of the gels prepared in Section 3.9.1 was determined using a centrifugation technique (Kinsella, 1984). The gels were centrifuged at (10,000Xg/15 min) and the supernatants, that separated were measured. Water holding capacity was expressed as the water retained in the residue after centrifugation. All measurements were done in triplicate.

3.9.4 Rheological Properties of Protein Co-precipitates

The samples mentioned in Section 3.9.1 were subjected to rheological properties. Protein samples (W/V) were dispersed in distilled water and stirred on magnetic stirrer to allow complete hydration of the proteins. The dispersions were adjusted to pH 8 with NaOH (2N). The rheological properties of the dispersions gels were determined using a rheometer (TA instruments, Advanced Rheometer, AR 2000, Great Britain). The sample dispersions (15ml) were analyzed as described by Lucey et al. (1997). The measuring geometry consisted of two coaxial cylinders, and the gap between the two plates was set to 1mm. The dispersions of samples were heated from 25 to (95°C/35 min) at a rate of 2°C/min. Results were expressed as the storage modules (G′) and loss modules (G″).

3.9.4.1 Denaturation and Gelation Start Temperature of Protein Co-precipitates

Denaturation start temperature (DST) was defined as that temperature at which the increase in viscoelasticity was not a significant due to endothermic transition, as heating increase (Jacoba et al., 2002). The gelation and denaturation start temperatures were determined by heating the dispersions as described in Section 3.9.4, the change in viscoelasticsity was measured as a function of increasing temperature. The gelation start temperatures (GST) were obtained at the temperatures at which the viscosity of the dispersion sharply increased (Shim and Mulvaney, 2001).

3.9.4.2 Effect of Protein Concentration on Rheological Properties

8%, 12% and 16% (W/V) of protein samples mentioned in Section 3.9.1 were dispersed in distilled water and adjusted to pH 8 with NaOH (2N). The dispersions of samples were analyzed as described in Section 3.9.4.

3.9.4.3 Effect of pH on Rheological Properties

Suspension (8%) of the protein samples used in Section 3.9.1, were prepared by mixing the protein co-precipitates with distilled water and adjusting pH to 5, 8, and 11 by adding either HCl (2N) or NaOH (2N). The dispersions of samples were analyzed as described in Section 3.9.4.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. PROTEIN CONTENT, ASH CONTENT AND YIELD OF CO-PRECIPITATES

Table 4.1 shows the protein contents, the ash contents and the yields of whey protein precipitates, soybean protein precipitates and protein co-precipitates prepared by different extraction and co-precipitation methods. Protein co-precipitates prepared by NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling treatments from MP and ME had the highest protein contents (85%-90%) followed by NaCl-NaOH/IEP-Cooling and NaOH-Papain/Cooling from MP and ME (50%-65%); NaCl-NaOH/Cooling MP and ME co-precipitates showed the lowest percentage of protein content (40%-45%). The lower protein contents of the co-precipitate extracted with NaCl-NaOH was related to the higher ash contents. It is likely that the use of NaCl in the extraction medium resulted in the presence of salt in the co-precipitates and was reflected in the high ash contents. The yields of protein co-precipitates were higher than those precipitated separately from whey protein precipitate and soybean protein precipitate. Thompson (1977) reported that the rapeseed-whey protein co-precipitates contained higher protein contents and yields than rapeseeds isolate precipitatets and whey protein precipitate individually.

The ME:NaOH/IEP-Heating-Cooling treatment gave the highest yield (45%) and protein content (90%). From the standpoint of yield; protein content and ash content, the co-precipitate ME: NaOH/IEP-Heating-Cooling is superior, followed by MP:NaOH/IEP-Heating-Cooling, then by both ME: and MP:NaOH/IEP-Cooling.

Table 4.1: Protein and Ash Contents and Yields of Whey Protein Precipitates, Soybean Protein Precipitates and Whey-Soybean Protein Co-precipitates.

Whey				***Soybean***			***Whey-Soybean Protein Co-precipitates***					
Method of Preparation	Ash%	*Protein %	**Yield %	Ash%	*Protein %	**Yield %	Ash (MP) %	*Protein (MP)%	**Yield (MP)%	Ash% (ME)	*Protein (ME)%	**Yield (ME)%
NaOH/Cooling	No	No	No	1.5±0.3	50	0.7±0.1	1.5±0.2	56	0.79±0.061	1.8±0.1	60	0.6±0.02
NaCl-NaOH/Cooling	14±2.3	38	0.61±0.08	15±2	40	0.64±0.12	11±0.2	40	0.42±0.093	14±0.3	42	1.6±0.24
NaOH-Papain /Cooling	No	No	No	1.2±0.2	45	0.53±0.13	7±1	62	0.54±0.0.31	1.6±0.2	52	0.52±0.03
NaCl-NaOH/IEP-Cooling	35±1.5	38	4.9±1.7	32±0.5	50	12±0.25	34±1.5	65	14±1.04	36±2	60	31±1.9
NaOH/IEP-Cooling	2±0.4	31	0.96±0.03	1.7±0.3	60	12.6±0.42	2.8±1	85	28±1.02	1.9±0.3	85	29±0.88
NaOH/ IEP-Heating-Cooling	2.3±0.5	32	6.1±0.35	1.5±0.2	65	26±1.95	2.2±1	90	35±0.85	3.6±0.5	90	45±6

*% Protein Content: Kjeldahl (N X Factor).
**Yield Based on Protein Content: %Protein Content X ((Weight of Protein Precipitates and Protein Co-precipitates)/sample Weight) X 100%).

The use of the mixed extract (ME) from whey and soybean was superior to the mixed powder (MP) in the NaOH/IEP-Heating-Cooling co-precipitate. In addition, the heating step in the co-precipitation for the mixed extract resulted in substantially higher yield of co-precipitate compared to the absence of heat (Table 4.1).

4.2 FACTORS AFFECTING ON THE YIELD OF PROTEIN CO-PRECIPITATES

4.2.1 Effect of Chelating Agents

Table 4.2 shows the yields of MP:NaOH/Cooling protein co-precipitates obtained with Na-HEXA, Ca-EDTA, and a mixture of Na-HEXA and Ca-EDTA. Ca-EDTA. The use of the mixture of Na-HEXA and Ca-EDTA resulted an increase in co-precipitate yield. The highest increase was observed with a mixture of Ca-EDTA and Na-HEXA at 0.5% and above, followed by Ca-EDTA at concentration 1.5%. Kosaric and Ng (1983) reported that the yield of milk protein co-precipitates increased with increase concentration of $CaCl_2$ (1-1.5%).

Table 4.3 shows the protein and ash contents and the protein yields for the following protein co-precipitates: MP: and ME:NaOH/IEP-Heating-Cooling and MP: and ME:NaOH/IEP-Cooling with Ca-EDTA, Na-HEXA, and mixture of Ca-EDTA and Na-HEXA. The MP: and ME:NaOH/IEP-Heating-Cooling co-precipitates obtained with the mixture of Ca-EDTA and Na-HEXA showed the highest protein contents. The use of the Ca-EDTA-Na-HEXA mixture resulted in co-precipitates of similar protein contents in the controls but with higher ash contents. The results suggest that the use of Na-HEXA contributes to the high ash contents of the co-precipitates.

Table 4.2: Effect of Chelating Agents on Yield of MP:NaOH/Cooling Co-precipitate.

Concentration of Chelating Agents %	*Protein Yield% Na-HEXA	*Protein Yield% Ca-EDTA	*Protein Yield% Mixture of Na-HEXA and Ca-EDTA 1:1
0	0.6±0.14	0.6±0.14	0.6±0.14
0.5	0.23±0.05	0.9±0.44	2.2±0.07
1.5	0.5±0.06	1.9±0.3	2.5±0.02
2.5	0.6±0.06	1.1±0.2	2.6±0.05

Yield on Wt Basis=((Weight of Protein Co-precipitates)/Sample Weight) X 100%).
* % Yield on Protein Content: Protein Content (60%) X Yield on Weight Basis.

Table 4.3: Effect of Chelating Agents on Protein Contents, Ash Contents and Yields of Protein Co-precipitates.

Control				***Na-HEXA***			***Ca-EDTA***			***Ca-EDTA and Na-HEXA***		
Protein Co-precipitate	Ash%	Protein %	*Yield %	Ash%	Protein %	*Yield %	Ash%	Protein %	*Yield %	Ash%	Protein %	*Yield %
NaOH/IEP-Cooling (MP)	2±0.05	88	27±0.61	12±0.37	70	25±0.285	0.5±0.12	60	13±0.415	11±0.15	83	27 ±0.29
NaOH/IEP-Cooling (ME)	2±0.1	80	28±0.43	15±0.20	80	28±0.213	0.7±0.2	70	10±0.56	14±1.64	87	35±1.67
NaOH/IEP-Heating-Cooling(MP)	2±0.1	85	29±0.43	12±1.5	75	40±3.12	1.4±0.1	70	39±1.23	11±0.5	84	48±0.13
NaOH/IEP-Heating-Cooling (ME)	3±0.35	90	42±2.31	14±1.8	80	40±1.5	1.6±0.6	65	41±2.5	13±0.7	89	48±1.12

* % Yield Based on Protein Content: %Protein Content X ((Weight of Protein Co-precipitates)/Sample Weight) X 100%).

The highest yield (48%) was obtained with the MP: and ME:NaOH/IEP-Heating-Cooling with the mixture of Ca-EDTA and Na-HEXA. Ca-EDTA and Na-HEXA individually did not increase yield compared to the controls, except for the co-precipitates obtained with the Ca-EDTA MP:NaOH/IEP-Heating-Cooling which showed higher yield (39%) compared with the control (29%).

4.2.2 Effect pH of Precipitation on Yield MP:NaOH/IEP-Heating-Cooling Co-precipitate

Table 4.4 shows the effect of pH of precipitation on the protein yields of protein co-precipitates; the yield increased from 25%, 30%, and 34% with increase from pH 3.5, 4 and 4.5 respectively, then decreased from 33% to 24% with increase from pH 5 to 6 respectively. The effect of pH on protein yield was related directly to the effect of yield in protein content but not weight yield. Berardi and Cherry (1981) found that 95% of protein in plant co-precipitates was recovered at pH 2.5. Youssef et al. (1995) also reported that the 92% of protein obtained at pH 4.6 in plant protein co-precipitates.

4.2.3 Effect of Precipitation Temperature on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate

Table 4.5 shows the effect of precipitation temperature on the yield of protein co-precipitates, the yield of protein increase gradually from 25%-35% as the precipitation temperature was increased from 60°C to 98°C. Kosaric and Ng (1983) reported that the recovered protein yield of milk protein co-precipitates increased rapidly with increasing temperature.

Table 4.4: Effect of pH of IEP on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate.

pH	Protein Content%	*Yield on Wt Basis%	**Yield on Protein Content%
3.5	60	42±0.3	25
4	70	43±0.5	30
4.5	85	40±0.2	34
5	83	40±0.7	33
6	60	40±0.7	24

*Yield on Weight Basis=((Weight of Protein Co-precipitates)/Sample Weight) X 100%).
** % Yield on Protein Content: Protein Content X Yield on Weight Basis.

Table 4.5: Effect of Precipitation Temperature on Yield of MP:NaOH/IEP-Heating-Cooling Co-precipitate.

Temperature °C	*Yield on Wt Basis%	**Yield on Protein Content%
60	29±1.1	25
70	34±2.4	29
80	35±2.4	30
90	38±1.2	32.3
98	41±1	35

*Yield on Weight Basis=((Weight of Protein Co-precipitates)/Sample Weight) X 100%).
** % Yield on Protein Content: Protein Content (85%) X Yield on Weight Basis.

4.3 POLYACRYLAMIDE GEL ELECTROPHORESIS FOR PROTEIN CO-PRECIPITATES

4.3.1 Native-PAGE

Figures 4.1 and 4.2 show the native-PAGE patterns of the protein precipitates from whey powder, soybean flour and the following protein co-precipitates: MP: and ME:NaOH/Cooling, NaOH-Papain/Cooling, NaOH/IEP-Cooling, NaOH/IEP-Heating-Cooling, NaCl-NaOH/Cooling and NaCl-NaOH/IEP-Cooling. The relative migrations of the identified bands are shown in Table 4.6. The protein from soybean powder shows an aggregate at the top of running gel and glycinin, β-conglycinin and 15S with relative migration of 0.27, 0.35 and 0.17, respectively; protein from from whey powder showed bands α-lactalbumin, β-lactoglobulin, BSA, and dimer β-lg with relative mobility 0.75, 0.90, 0.58 and 0.68, respectively. All co-precipitates showed the presence of the soybean proteins glycinin, β-conglycinin and 15S with relative migration 0.26, 0.32 and 0.16 respectively, and the whey proteins with bands α-lactalbumin, β-lactoglobulin, BSA and dimer β-lg with relative migration 0.74, 0.92, 0.58 and 0.68 respectively. In addition two new bands with relative migration 0.63 and 0.78 were observed.

Figures 4.3 and 4.4 show two new bands with relative migration (0.58 and 0.87) in supernatants from NaOH/IEP-Heating-Cooling and NaOH/IEP-Cooling co-precipitates; these were not identified in both soybean and whey proteins (Table 4.7).

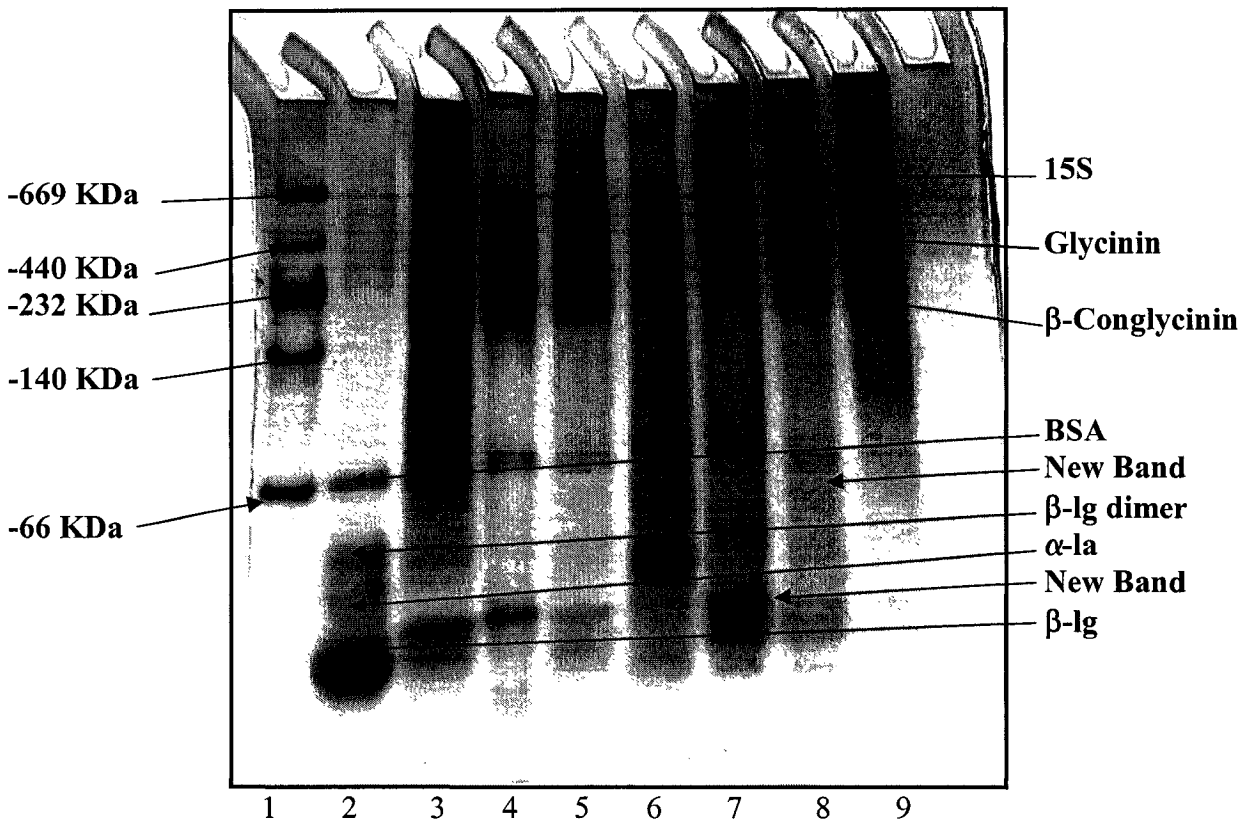


Figure 4.1: Native-PAGE Electropherogram of MP:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

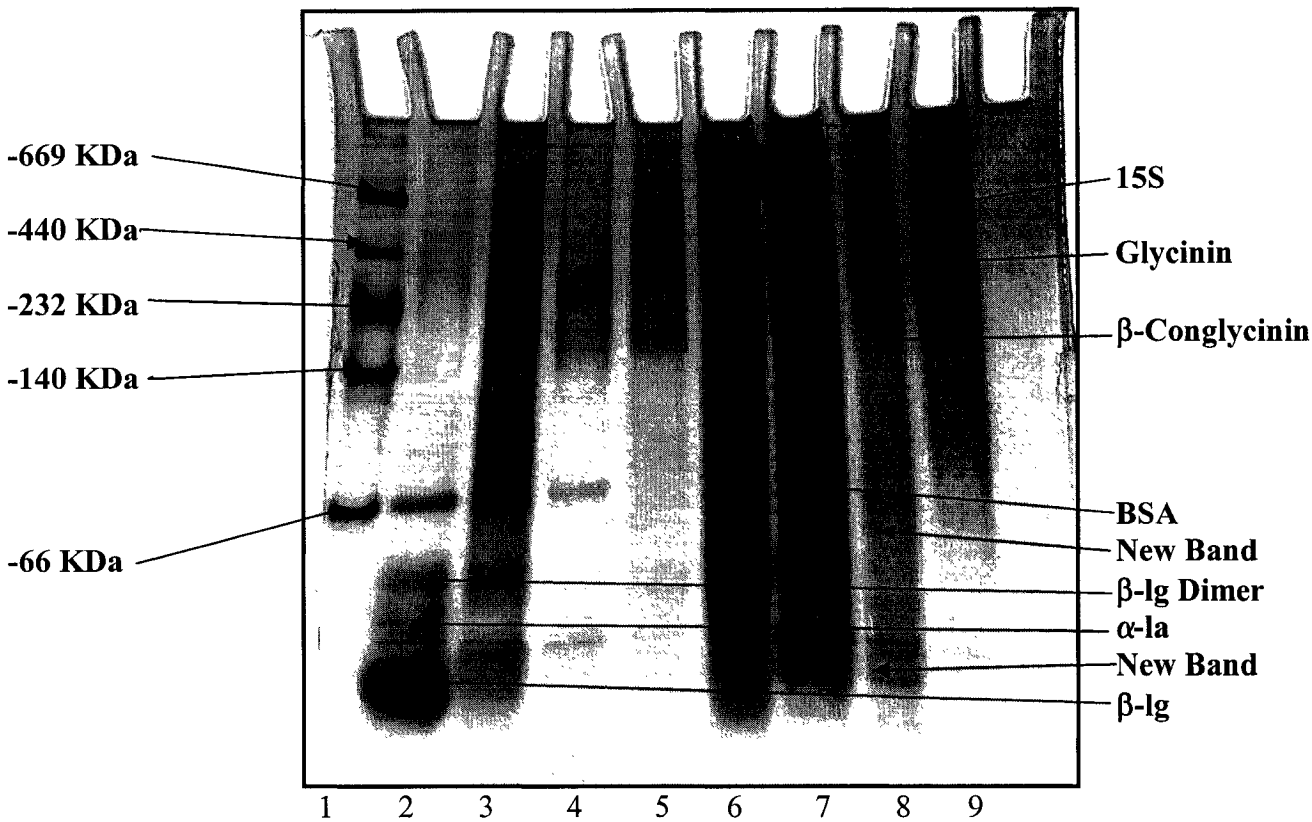


Figure 4.2: Native-PAGE Electropherogram of ME:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

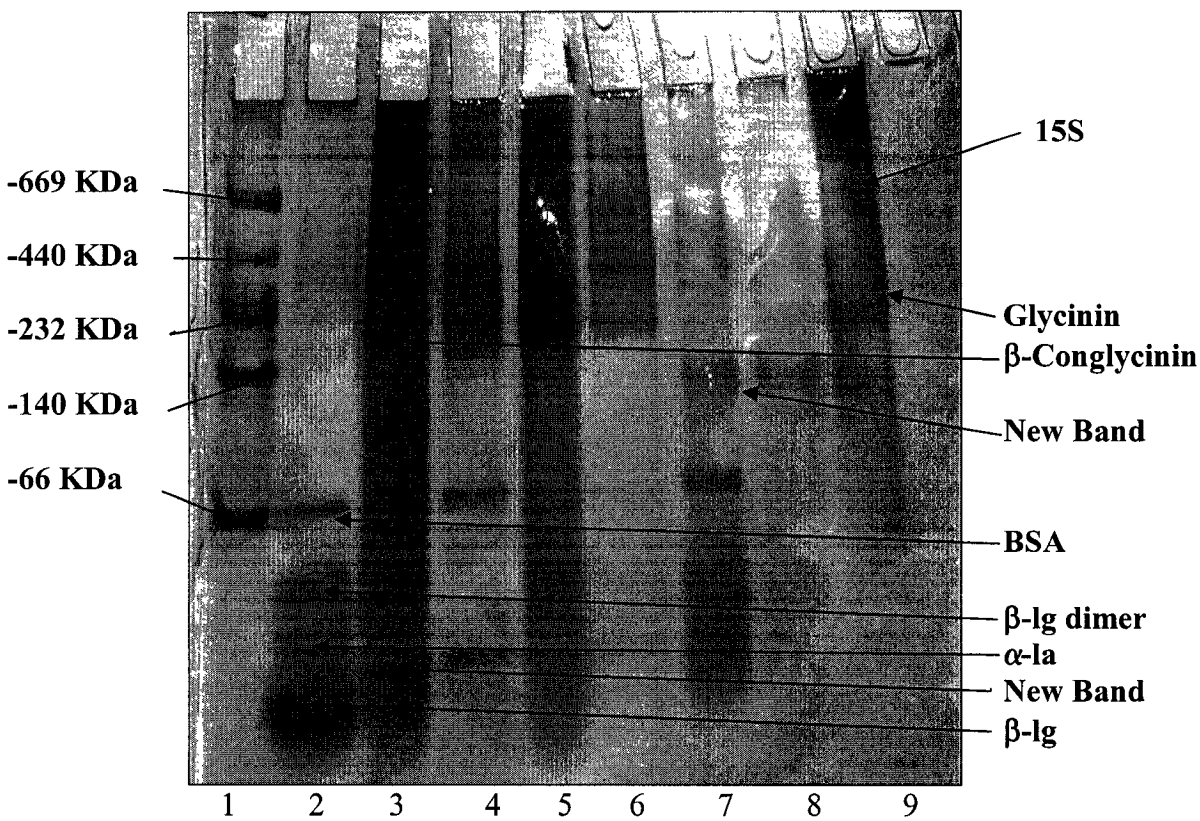


Figure 4.3: Native-PAGE Electropherogram of supernatants for MP:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

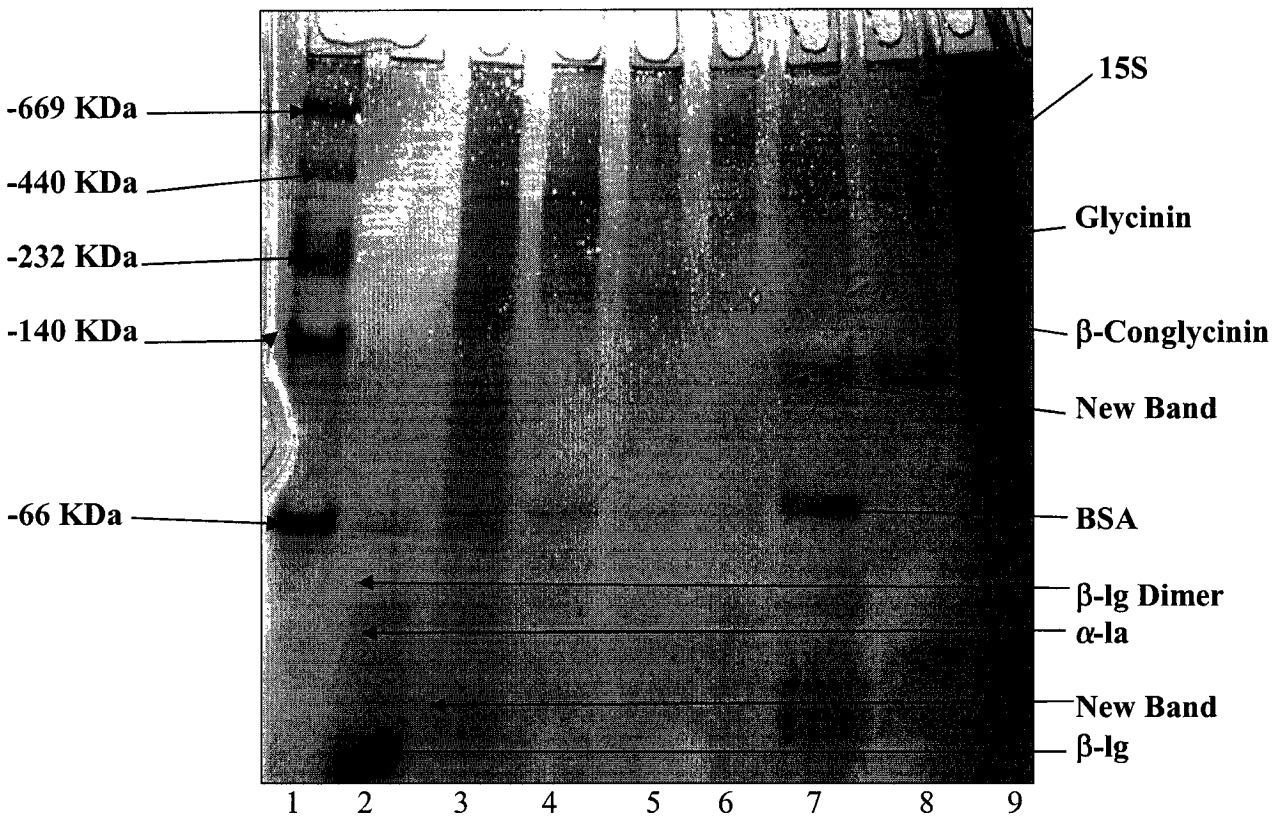


Figure 4.4: Native-PAGE Electropherogram of supernatants for ME:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

Table 4.6: Relative Migration (RM) of Major Components Fractions of Whey Protein Powder, Soybean Protein Flour and Protein Co-precipitates.

Protein Fractions	*(RM) of Protein Fractions in Co-precipitates	*(RM) Of Protein Fractions from Soybean	*(RM) of Protein Fractions from Whey
(15S)	0.16	0.17	
Glycinin	0.26	0.27	
B-Conglycinin	0.32	0.35	
BSA	0.58		0.58
NEW BAND	0.63		
Dimeric β-lg	0.68		0.68
α-la	0.74		0.75
NEW BAND	0.78		
β-lg	0.92		0.90

*** RM: Relative Migration: Distance Migrated by Protein/Distance Migrated by Dye.**

Table 4.7: Relative Migration (RM) of Major Components Fractions for Whey Protein Powder, Soybean Protein Flour and Supernatants of Protein Co-precipitates.

Protein Fractions	*(RM) of Protein Fractions in Supernatant	*(RM) of Protein Fractions from Soybean	*(RM) of Protein Fractions From Whey
(15S)	0.14	0.13	
Glycinin	0.21	0.24	
B-Conglycinin	0.32	0.35	
NEW BAND	0.58		
BSA	0.61		0.63
Dimeric β-lg	0.76		0.79
α-la	0.81		0.84
NEW BAND	0.87		
β-lg	0.93		0.96

*** RM: Relative Migration: Distance Migrated by Protein/Distance Migrated by Dye.**

The proteins identified in the supernatants contain similar components when compared to the protein co-precipitates. The presence of new bands in both the supernatants and the protein co-precipitates suggests that there are interactions between whey and soybean proteins during the preparation and extraction of the co-precipitates. The presence of the new bands in the supernatants also suggests that these proteins were not due only to the precipitation procedure.

4.3.2 SDS-PAGE ELECTROPHORESIS

4.3.2.1 Identification of Subunits for Protein Co-precipitates

Figures 4.5 and 4.6 show SDS-PAGE patterns of the whey powder, soybean powder and the following protein co-precipitates: MP: and ME:NaOH/Cooling, NaOH-Papain/Cooling, NaOH/IEP-Cooling, NaOH/IEP-Heating-Cooling, NaCl-NaOH/Cooling and NaCl-NaOH/IEP-Cooling. All protein co-precipitates showed 20 protein subunits, which corresponds to subunits of whey proteins and of soybean proteins; the only exception was the NaOH-Papain/Cooling co-precipitates which showed bands that represent hydrolysis products of the proteins. The molecular weights of the subunits shown in Table 4.8 confirm the identity of the proteins in the co-precipitates. The ME protein co-precipitates gave protein electrophoretic patterns similar to those of MP protein co-precipitates. Figures 4.7 and 4.8 show the same bands in the supernatants as well as the co-precipitates obtained after precipitation of the protein

The native-PAGE results (Section 4.3.1) showed new bands in the protein co-precipitates; however no new bands were observed in the SDS-PAGE results. This suggests that the new protein, which were formed by interaction of whey proteins and

soybean proteins during the preparations of the co-precipitates were dissociated into whey and soybean proteins subunits in the presence of SDS.

4.3.2.2 SDS PAGE of Protein Co-precipitates Obtained with Using Chelating Agents

Figure 4.9 shows the SDS-PAGE patterns of the protein co-precipitates MP: NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling obtained with use of Ca-EDTA and NA-HEXA, no major differences were observed when the SDS-PAGE results for the NaOH/IEP-Cooling, NaOH/EP-Heating-Cooling and NaOH/Heating-Cooling co-precipitates treated with Ca-EDTA, Na-HEXA and mixture of Ca-EDTA and Na-HEXA were compared to the SDS-PAGE results of the control samples.

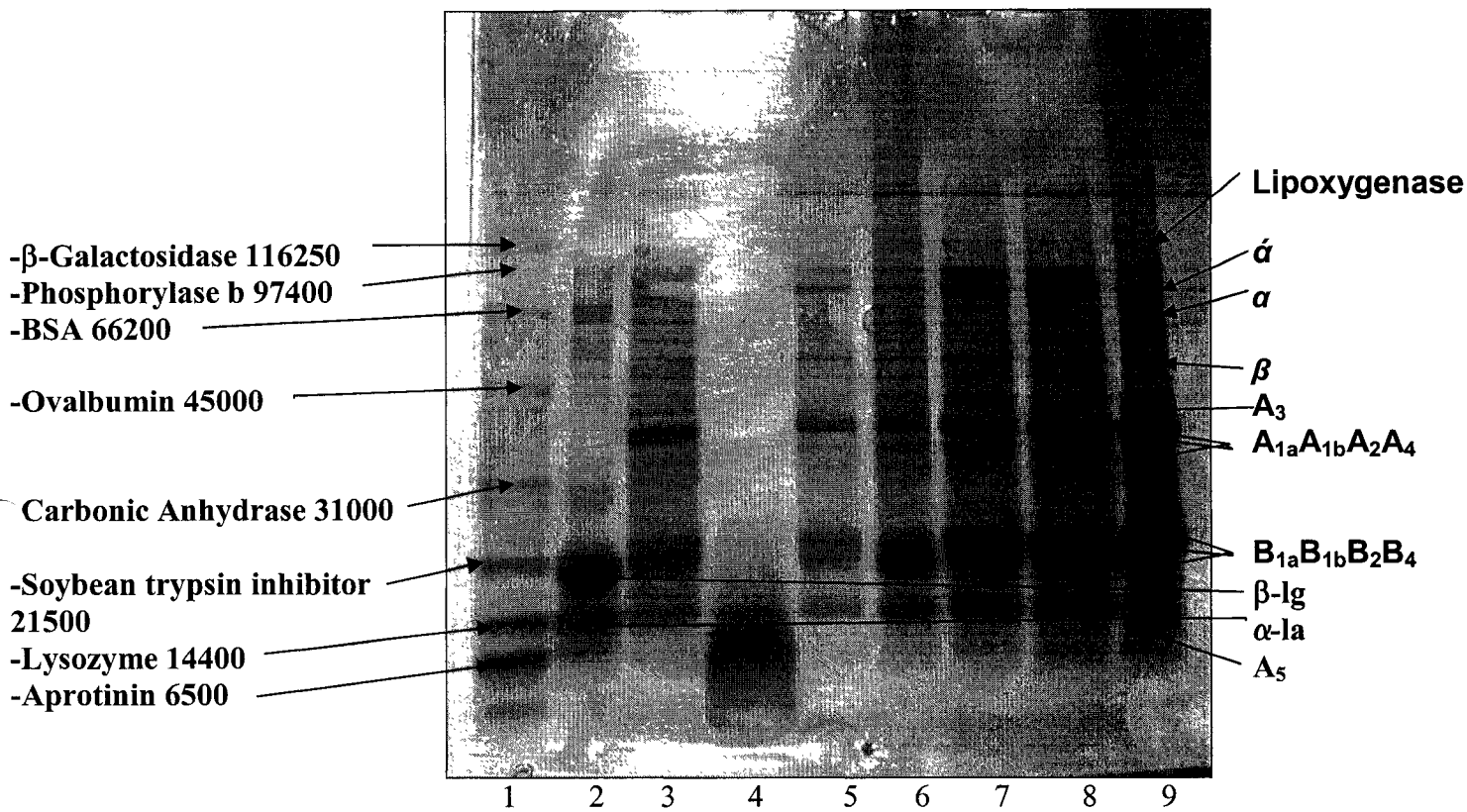


Figure 4.5: SDS-PAGE Electropherogram of MP:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

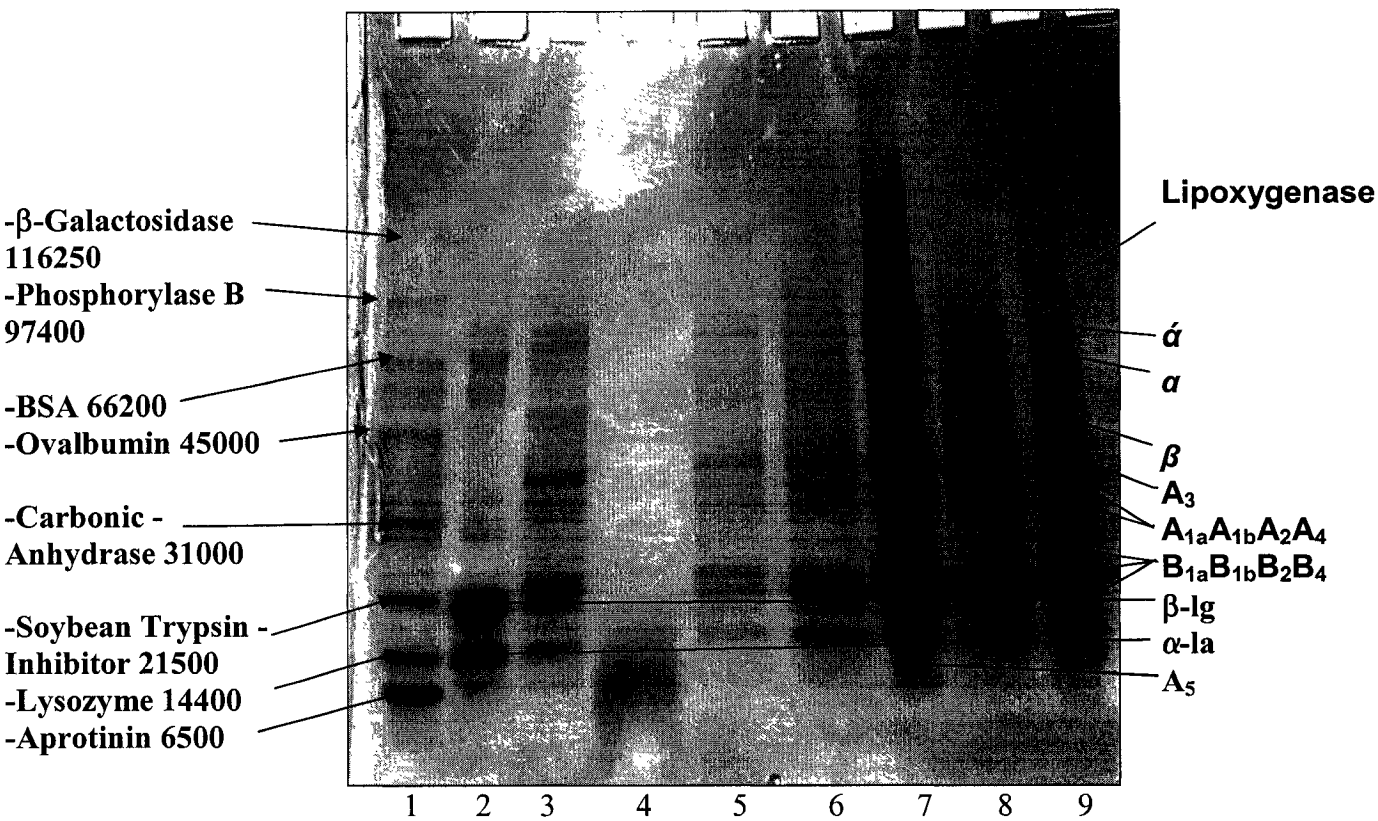


Figure 4.6: SDS-PAGE Electropherogram of ME:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

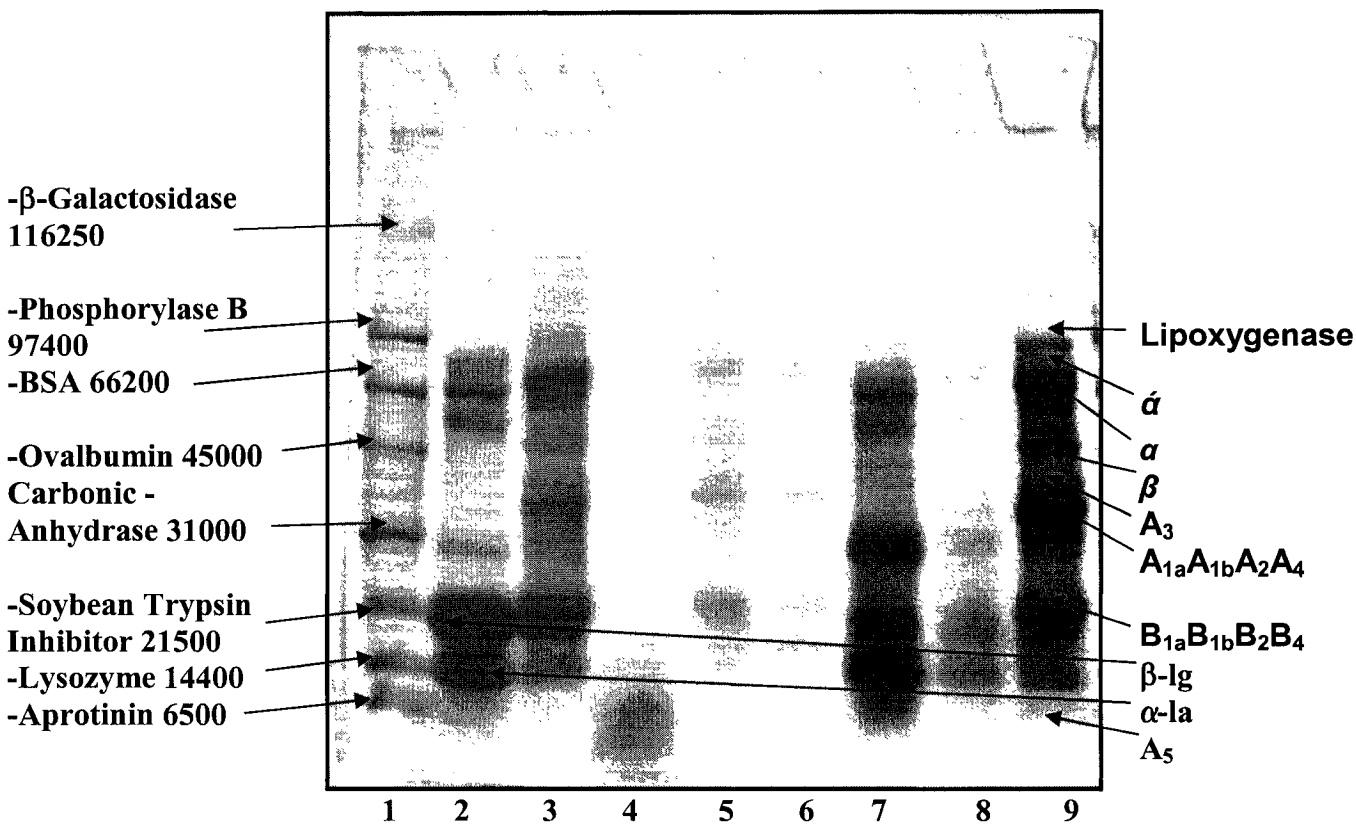


Figure 4.7: SDS-PAGE Electropherogram of supernatants for MP:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaOH/IEP-Cooling, (5); NaOH/IEP-Heating-Cooling, (6); NaCl-NaOH/Cooling, (7); NaCl-NaOH/IEP-Cooling, (8); Soybean protein, (9).

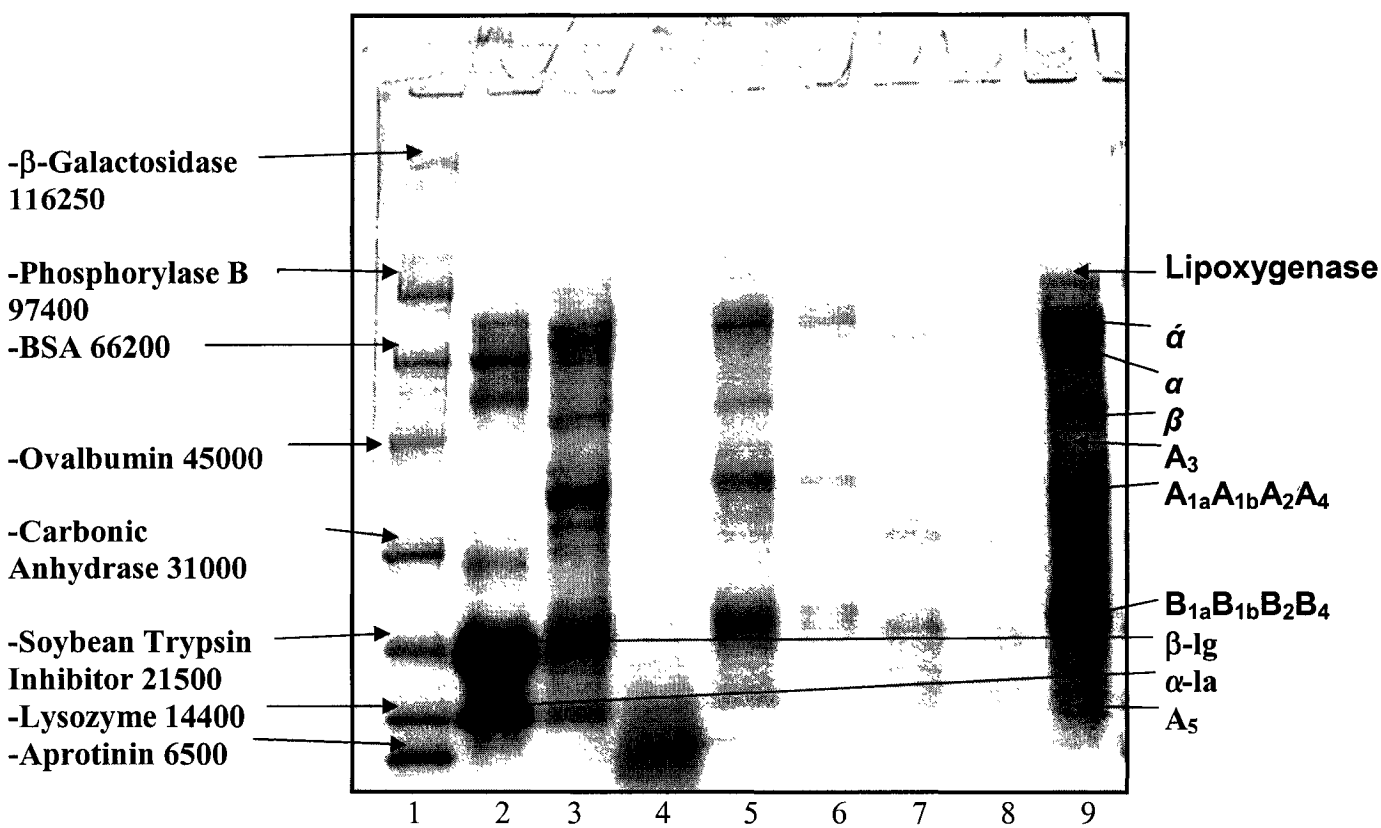


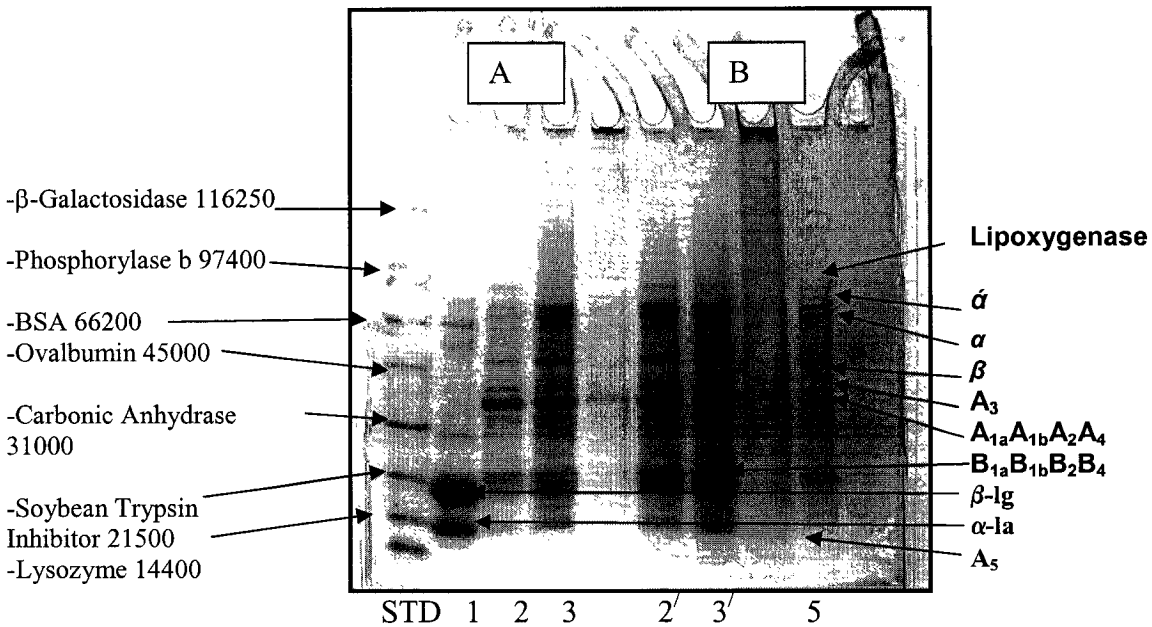
Figure 4.8: SDS-PAGE Electropherogram of supernatant for ME:protein co-precipitates, Std, (1); Whey protein, (2); NaOH/Cooling, (3); NaOH-Papain/Cooling, (4); NaCl-NaOH/Cooling, (5); NaCl-NaOH/IEP-Cooling, (6); NaOH/IEP-Cooling, (7); NaOH/IEP-Heating-Cooling, (8); Soybean protein, (9).

Table 4.8: Estimated Molecular Weight of Subunits (Da) of Major Components Fractions of Whey Protein, Soybean Protein and Protein Co-precipitates.

Protein Fractions	*MW (Da) of Fractions of Proteins in Co-precipitates	MW. (Da) of Fractions of Proteins from Soybean	MW (Da) of Fractions of Protein from Whey	MW (KDa) Of Soybean And Whey Proteins Reported in the Literatures
Aggregates	102417	102417		100000_A
Lipoxygenase	97400	97400		93330_B
ά	83000	83000		82200_C
α	68600	68600		70630_D
BSA	67407		67407	66646_E
B	52572	52572		52000_F
A_3	42790	42790		42000_G
A_{1a} A_{1b} A_2 A_4	38368, 35421, 33948	38368, 35421, 33948		38000_H
Agglutinin (H_I and H_{II})	27607, 25571, 24215	27607, 25571, 24215		30000_I
B_{1a} B_{1b} B_2 B_4	21500, 20486, 19472	21500, 20486, 19472		20650_J
Globulin (L_I andL_{II})	18457	18457		18000_K
β-lg (B,A)	18000		18000	18277_L
A-la	14400		14400	14175_M
A_5	10077	10077		10000_N

A.Petruccelli and Anon, (1995); B. Sathe et al., (1987); C. Sathe et al., (1987); D. Sathe et al., (1987); E. Loo et al., (1991); F. Nielsen , (1985b); G. Moreira et al., (1979); H. Utsumi and Kinsella., (1985); I. Petruccelli and Anon, (1995); J. Sathe et al., (1987); K. Sathe et al., (1989); L. Morgan et al., (1997); M. Smith et al., (1990); N. Sathe et al., (1987).

*The results are same to other co-precipitates except NaOH-Papain/Cooling co-precipitates.



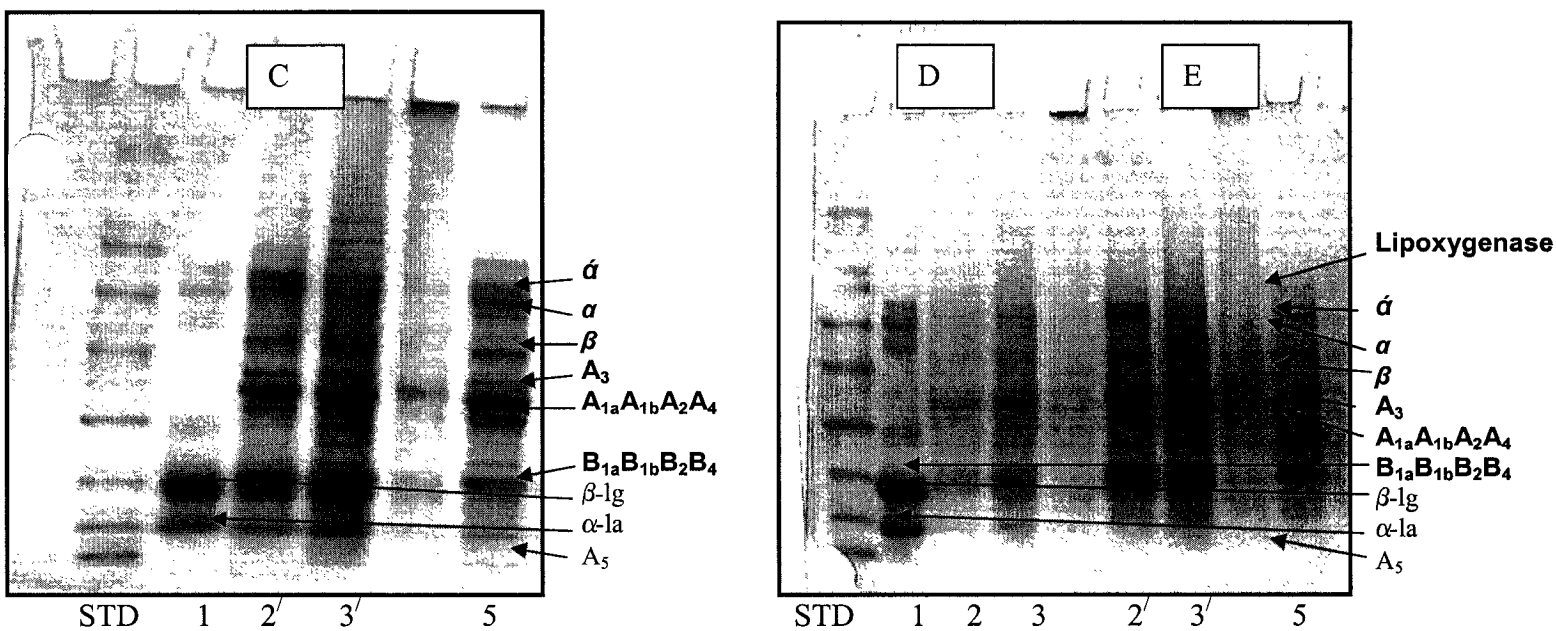


Figure 4.9: Electropherogram of SDS PAGE for MP:protein Co-precipitate in the presence of (A) Mixture of Ca-EDTA and Na-HEXA; (B, D) Control; (C) Na-HEXA; (E) Ca-EDTA, (1), Whey protein; (2, 2′), NaOH/IEP-Cooling; (3, 3′), NaOH/IEP-Heating-Cooling; (5), Soybean protein.

4.4 MICROSCOPY OF PROTEIN CO-PRECIPITATES

Figure 4.10 shows the microscopic structure of the following samples: MP:NaCl-NaOH/IEP-Cooling ME:NaCl-NaOH/IEP-Cooling for protein co-precipitates, whey protein precipitates:NaCl-NaOH/IEP-Cooling and soybean protein precipitates:NaCl-NaOH/IEP-Cooling. Both MP: and ME: (Figure 4.10) the results showed a thick strand, more and a large cluster of a network structure which was different from the particulate structure observed with the soybean protein precipitate and the whey protein precipitate.

Figure 4.11 shows the microscopic structure of the following samples: MP:NaOH/IEP-Cooling, ME:NaOH/IEP-Cooling, whey protein precipitates:NaOH/IEP-Cooling, and soybean protein precipitates:NaOH/IEP-Cooling. The protein co-precipitates showed network structure that was different from the particulate structure observed with soybean protein precipitate:NaOH-Cooling and whey protein precipitate:NaOH/IEP-Cooling. Protein co-precipitates from MP:NaOH/IEP-Heating-Cooling showed somewhat structure similar to the network structures of protein co-precipitates from MP: and ME:NaOH/IEP-Cooling.

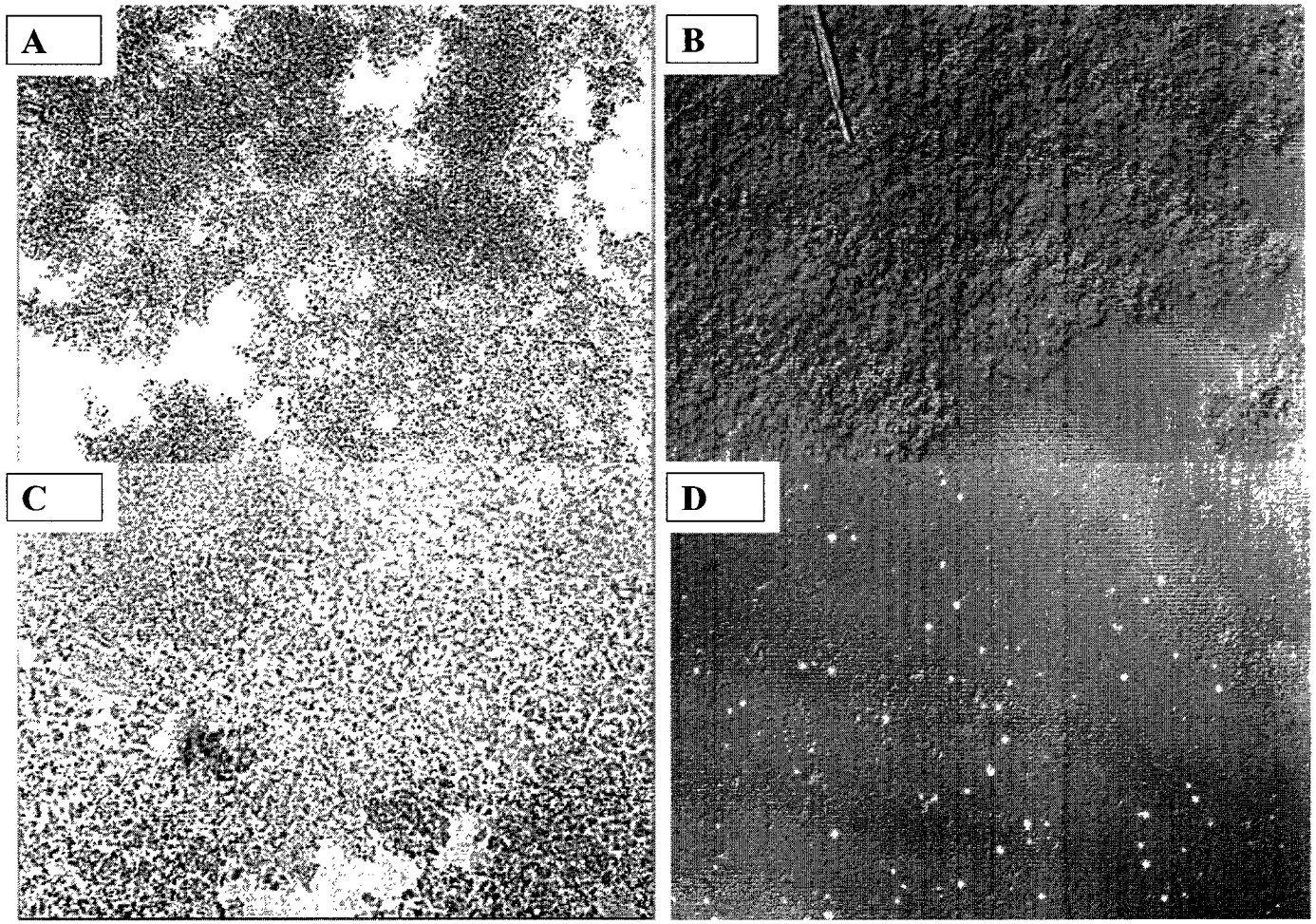


Figure 4.10: Micrographs (Mag.X1000) for whey protein precipitate, soybean protein precipitate and protein co-precipitates (A) Whey protein prepared by NaCl-NaOH/IEP-Cooling; (B) Protein co-precipitate prepared by MP:NaCl-NaOH/IEP-Cooling; (C) Soybean protein prepared by NaCl-NaOH/IEP-Cooling.; (D) Protein co-precipitate prepared by ME:NaCl-NaOH/IEP-Cooling.

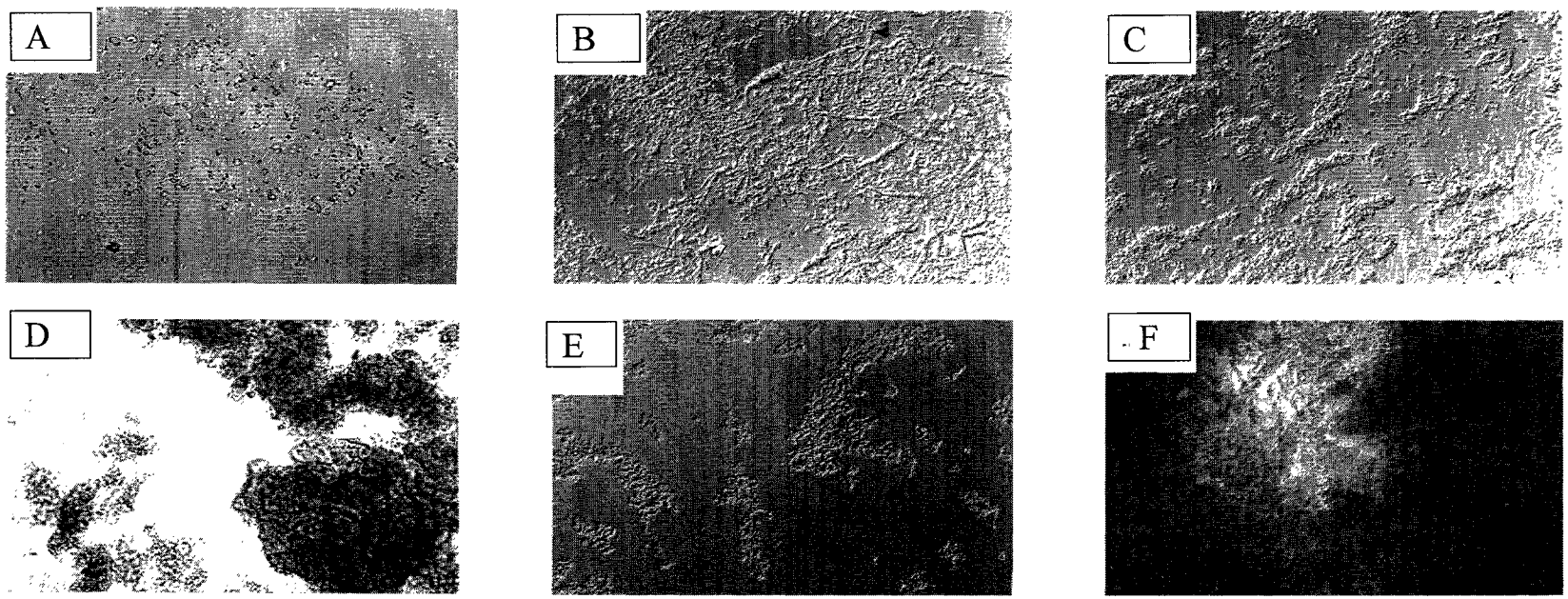


Figure 4.11: Micrographs (Mag.X1000) for whey protein precipitate, soybean protein precipitate and (MP: and ME:) of protein co-precipitates. (A) Whey protein prepared by NaOH/IEP-Cooling; (B) Protein co-precipitate prepared by MP:NaOH/IEP-Cooling ;(C) MP:Protein co-precipitate prepared by MP:NaOH/IEP-Heating-Cooling; (D) Soybean protein prepared by NaOH/IEP-Cooling; (E) Protein co-precipitate prepared by ME:NaOH/IEP-Cooling; (F) Protein co-precipitate prepared by ME:NaOH/IEP-Heating-Cooling.

4.5 GELATION AND RHEOLOGICAL PROPERTIES OF PROTEIN CO-PRECIPITATES

4.5.1 Denaturation Start Temperature and Gelation Start Temperature

Table 4.9 shows the denaturation start temperature (DST) and gelation start temperature (GST) for 8% whey protein precipitates:NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling, soybean protein precipitates:NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling, and MP: and ME:NaOH/IEP-Cooling, and MP: and ME:NaOH/IEP-Heating-Cooling co-precipitates. The MP:NaOH/IEP-Cooling showed the highest DST (58°C) followed by the whey protein precipitates:NaOH/IEP-Cooling (55°C), the soybean protein precipitate:NaOH/IEP-Cooling (52°C), and the protein co-precipitate ME:NaOH/IEP-Cooling with the lowest DST (49°C). The protein co-precipitate ME:NaOH/IEP-Heating-Cooling had a higher DST (54°C) than whey protein precipitate: and soybean protein precipitate:NaOH/IEP-Heating-Cooling (48°C), while protein co-precipitate MP:NaOH/IEP-Heating-Cooling had the lowest DST (33°C).

The highest GST was observed with MP:NaOH/IEP-Cooling co-precipitate; the co-precipitate also showed the highest DST. The lowest GST was observed with MP:NaOH/IEP-Heating-Cooling; this co-precipitate also showed the lowest DST. The whey protein precipitate:NaOH/IEP-Cooling had a higher GST (87°C) than soybean protein precipitates:NaOH/IEP-Cooling (83°C). The GST for both whey protein precipitate:NaOH/IEP-Heating-Cooling and soybean protein precipitate:NaOH/IEP-Heating-Cooling were (78°C) as shown in Table 4.9.

4.5.1.1 Effect of Protein Concentration on DST and GST

Figure 4.12 A shows the effect of concentration on the DST of the protein co-precipitates MP: and ME:NaOH/IEP-Cooling, and MP: and ME:NaOH/IEP-Heating-Cooling. In general, protein concentration had a little effect on DST, except in the case of the MP:NaOH/IEP-Cooling , which showed a sharp decrease in DST from 58°C to 43°C, when the concentration increased from 12% to 16%.

Figure 4.12 B shows the effect of concentration on the GST for protein co-precipitates MP: and ME:NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling. For protein co-precipitate MP:NaOH/IEP-Heating-Cooling, there was relatively effect of concentration on GST. The MP:NaOH/IEP-Cooling co-precipitate showed a sharp decrease in GST from 89°C to 76°C between 12% and 16% protein concentration; this correspond to the protein concentration which showed a sharp decrease in DST of the co-precipitate (Figure 4.12 A).

4.5.1.2 Effect of pH on DST and GST

The DST and GST for protein co-precipitates MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling were affected by pH (Figure 4.13 A), at pH 5 protein co-precipitate ME:NaOH/IEP-Heating-Cooling showed the highest DST(50°C) followed by MP:NaOH/IEP-Cooling (46°C), while ME:NaOH/IEP-Cooling and MP:NaOH/IEP-Heating-Cooling showed similar DST (44°C). At pH 8, the MP:NaOH/IEP-Cooling co-precipitate showed a high DST (58°C); MP:NaOH/IEP-Heating-Cooling showed the lowest DST (31°C).

Table 4.9: Denaturation Start Temperature (DST) and Gelation Start Temperature (GST) (˚C) for Whey Protein Precipitates, Soybean Protein Precipitates and Protein Co-precipitates.

Type	Whey		Soybean		Protein Co-precipitates NaOH/IEP-Cooling		Protein Co-precipitates NaOH/IEP-Heating-Cooling	
	NaOH/IEP-Cooling	NaOH/IEP-Heating-Cooling	NaOH/IEP-Cooling	NaOH/IEP-Heating-Cooling	**MP:**	**ME:**	**MP:**	**ME:**
Denaturation Start Temperature ˚C (DST)	55	48	52	48	58	49	33	54
Gelation Start Temperature ˚C (GST)	87	78	83	78	89	79	61	85

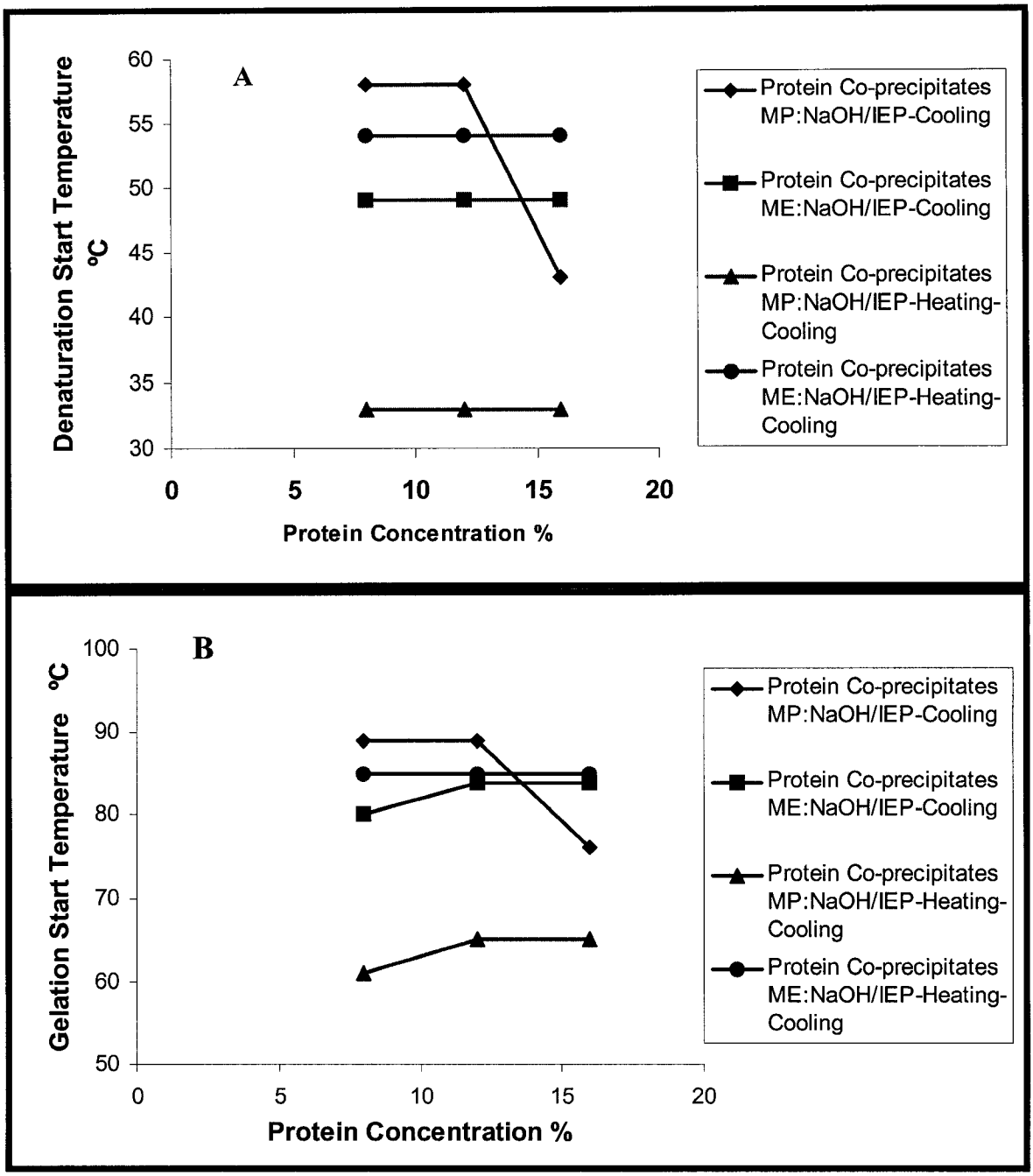


Figure 4.12: (A) Effect of concentration on DST for protein co-precipitates MP: and ME:NaOH/IEP-Cooling MP: and ME:NaOH/IEP-Heating-Cooling. (B) Effect of concentration on GST for protein co-precipitates from MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling.

The DST was similar for ME:NaOH/IEP-Cooling and ME:NaOH/IEP-Heating-Cooling (50°C). When the pH increased from 8 to 11, the higher DST (49-51°C) was observed for MP:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling, while ME:NaOH/IEP-Cooling showed a DST (44°C).

Figure 4.13 B shows the effect of pH on the GST of the protein co-precipitates. At pH 8 MP:NaOH/IEP-Cooling, ME:NaOH/IEP-Cooling and ME:NaOH/IEP-Heating-Cooling had higher GST (89, 81 and 87°C) than at pH 5 and pH 11. The GST MP:NaOH/IEP-Heating-Cooling at pH 8 (61°C) was lower GST than pH 5 and pH 11. On the other hand, at pH 5 the GST for MP: and ME:NaOH/IEP-Cooling and MP:NaOH/IEP-Heating-Cooling were similar (77°C); ME:NaOH/IEP-Heating-Cooling showed a slightly higher GST (80°C). At pH 11 MP:NaOH/IEP-Cooling had the highest GST (85°C) followed by ME:NaOH/IEP-Cooling and MP:NaOH/IEP-Heating-Cooling which showed similar GST (80°C), while ME:NaOH/IEP-Heating-Cooling showed a slightly lower GST (77°C).

4.5.2 Effect of Protein Concentration of Protein Co-precipitates on Gel Strength and Water Holding Capacity (WHC) of gels

In general, the gel strengths for protein co-precipitate were greatly increased with increasing concentration (Figure 4.14 A and Table 4.10); all protein co-precipitate gave firm gel structure at 16% concentration. At 8% there is no gel for whey protein precipitate:NaOH/IEP-Cooling and protein co-precipitate MP and ME:NaOH/IEP-Cooling.

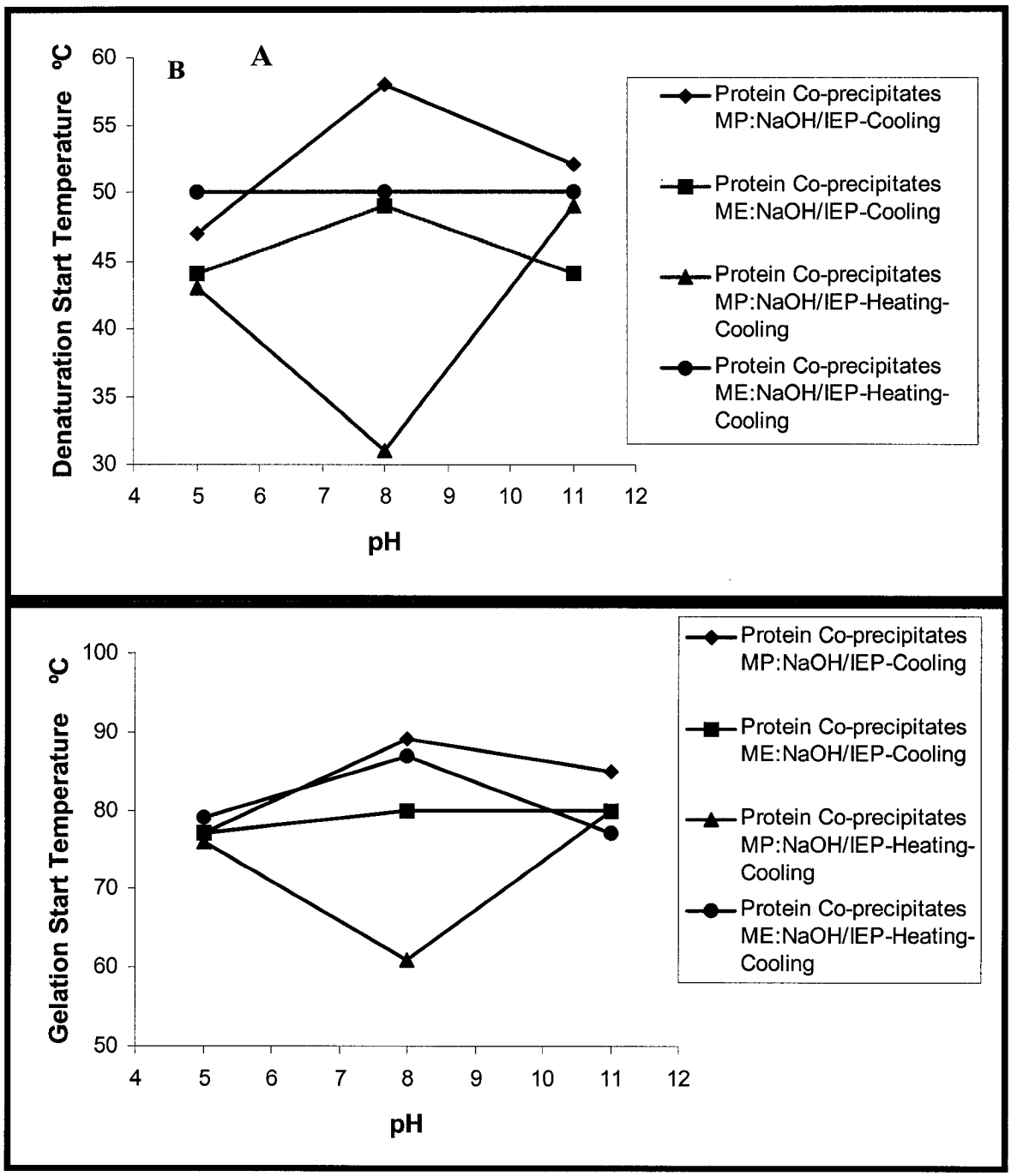


Figure 4.13: (A) Effect of pH on DST for protein co-precipitates MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling. (B) Effect of pH on GST for protein co-precipitates MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling.

The WHC for all gels increased with protein concentration (Figure 4.14 B and Table 4.10). Smith (1960) reported that soy isolate gels showed higher WHC than those from milk protein gels; Kebary (1993) reported similar results for WHC. Gels from protein co-precipitate MP:NaOH/IEP-Cooling and whey protein precipitate gels showed higher WHC than gels from soybean precipitates. Similar results were also observed when the gel from co-precipitates ME:NaOH/IEP-Cooling. WHC for gel from the soybean protein precipitate showed lowest value; these results in agreement with (Kosaric and Ng, 1983).

Figure 4.15 A and Table 4.10 show the gel strengths for whey protein precipitate, soybean protein precipitate and protein co-precipitate MP: and ME:NaOH/IEP-Heating-Cooling. At 8% ME:protein co-precipitate and soybean protein precipitate gels showed similar gel strengths. Similar results were observed for gel of MP:protein co-precipitates and whey protein precipitate. When the concentration increase to 12% gel from ME:protein co-precipitates showed the highest gel strengths followed by soybean protein precipitate gel and MP:protein co-precipitate. At 16% protein concentration, gel strengths were highest for ME:protein co-precipitate followed by gels from soybean protein precipitate, whey protein precipitate and MP:protein co-precipitate, respectively.

Figure 4.15 B and Table 4.10 show The concentration versus WHC of gels from whey protein precipitate, soybean protein precipitate, and MP: and ME:NaOH/IEP-Heating-Cooling protein co-precipitate. The gels from soybean protein precipitates show the highest WHC followed by gels from whey protein precipitate; the gels from protein ME:co-precipitate showed the lowest WHC at each concentration.

The gel strengths for the gels from NaOH/IEP-Cooling and NaOH/IEP-Heating-Cooling protein co-precipitate are shown in Figure 4.16 A and Table 4.10. At 8% and 12% protein concentration, no gel was observed for protein co-precipitate MP: and ME:NaOH/IEP-Cooling and MP:NaOH/IEP-Heating-Cooling. At 16% protein concentration, highest gel strengths were observed for gels from MP:NaOH/IEP-Cooling followed by ME:NaOH/IEP-Cooling, ME:NaOH/IEP-Heating-Cooling and MP:NaOH/IEP-Heating-Cooling, respectively. Figure 4.16 B and Table 4.10 show the WHC for gels from MP: and ME:NaOH/IEP-Cooling and gels from MP: and ME:NaOH/IEP-Heating-Cooling. There were no differences between the WHC of protein co-precipitate gels from MP: and ME:NaOH/IEP-Cooling. The gels from MP:NaOH/IEP-Heating-Cooling showed higher WHC than gels from ME:NaOH/IEP-Heating-Cooling. Gels MP: and ME:NaOH/IEP-Cooling showed higher WHC than gels from MP: and ME:NaOH/IEP-Heating-Cooling.

Table 4.10: Gel Strength (N) and Water Holding Capacity (WHC%) of Whey Protein Precipitates, Soybean Protein Precipitates and Protein Co-precipitates.

Preparation	Protein Co-precipitates NaOH/IEP-Cooling		Protein Co-precipitates NaOH/IEP-Heating-Cooling		Whey protein		Soybean protein	
Concentration %	WHC% MP	WHC% ME	WHC% MP	WHC% ME	WHC% NaOH/IEP-Cooling	WHC% NaOH/IEP-Heating-Cooling	WHC% NaOH/IEP-Cooling	WHC% NaOH/IEP-Heating-Cooling
8	95.5±0.71	95.4±1.18	35±1.3	35±2	95±0.8	46±2.5	88±1.7	48±5.3
12	98.4±0.34	98.5±0.09	53±2.1	47±1.5	98±0.4	53±1.1	92±0.5	69±4.5
16	99.8±0.08	100±0.0	62±2.2	55±2.4	100±0.05	67±0.8	94±0.27	84±0.5

Preparation	Protein Co-precipitates NaOH/IEP-Cooling		Protein Co-precipitates NaOH/IEP-Heating-Cooling		Whey protein		Soybean protein	
Concentration %	Gel Strength MP	Gel Strength ME	Gel Strength MP	Gel Strength ME	Gel Strength NaOH/IEP-Cooling	Gel Strength NaOH/IEP-Heating-Cooling	Gel Strength NaOH/IEP-Cooling	Gel Strength NaOH/IEP-Heating-Cooling
8	0	0	0	0.3	0	0	0.7	0.3
12	0.5	0.3	0.3	1.2	0	0	2.2	0.6
16	5	3	0.7	2.4	3.1	1.3	3	1.6

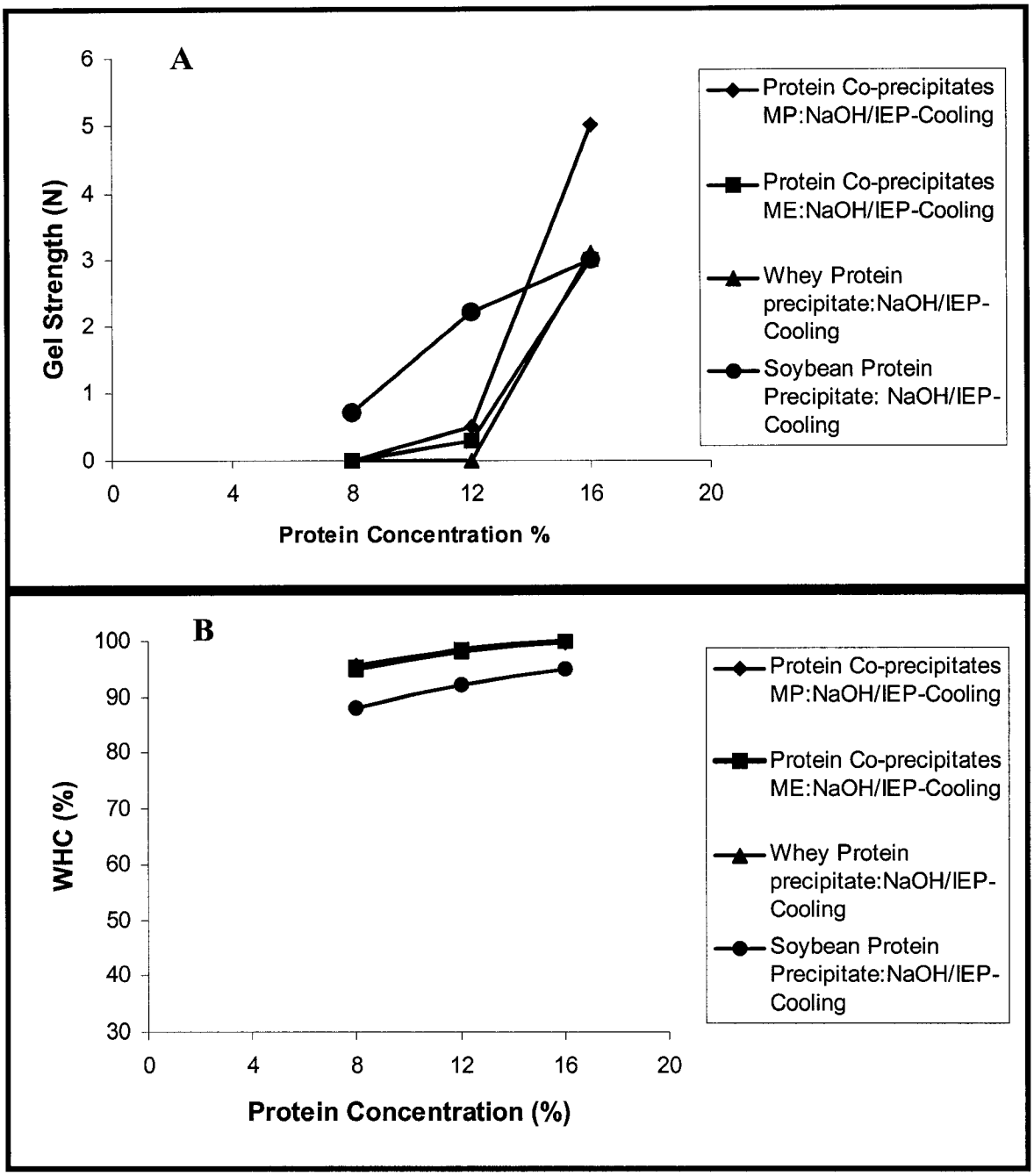


Figure 4.14: Effect of concentration on (A) Gel Strength and (B) Water Holding Capacity (WHC) for whey protein precipitates, soybean protein precipitates and protein co-precipitates gels MP: and ME:NaOH/IEP-Cooling.

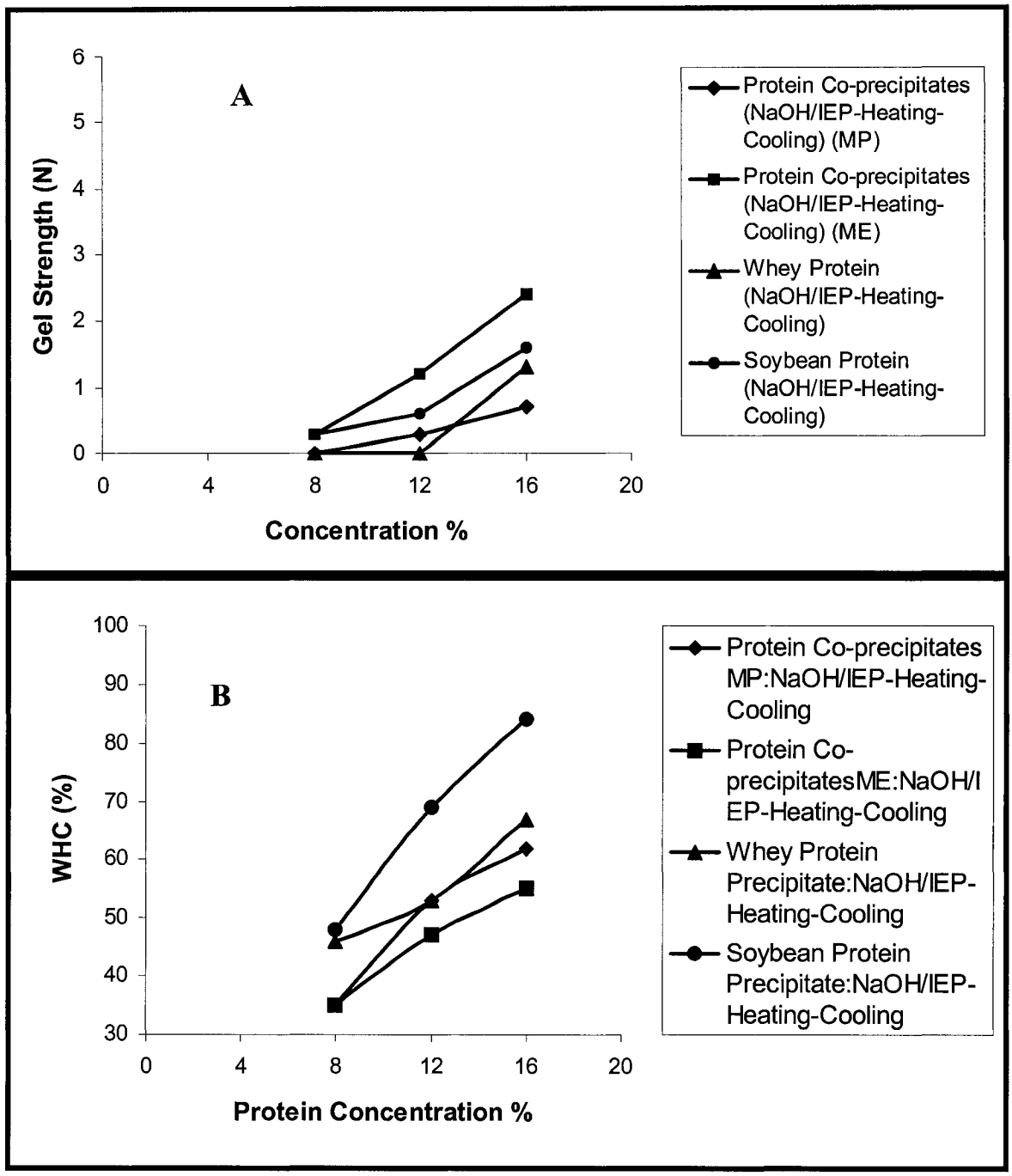


Figure 4.15: Effect of concentration on (A) Gel Strength and (B) Water Holding Capacity (WHC) for whey protein precipitates, soybean protein precipitates and protein co-precipitates gels MP: and ME:NaOH/IEP-Heating-Cooling.

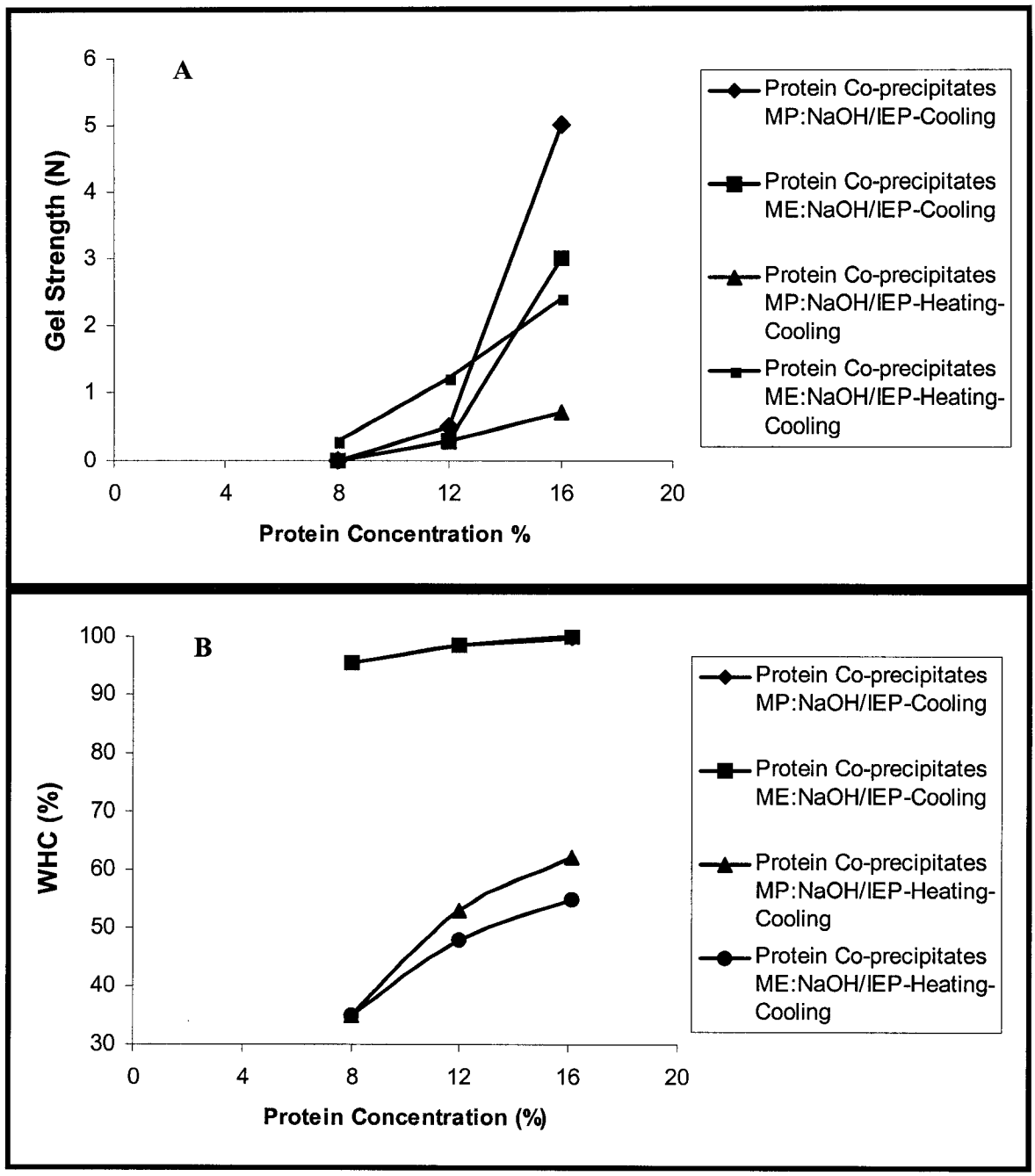
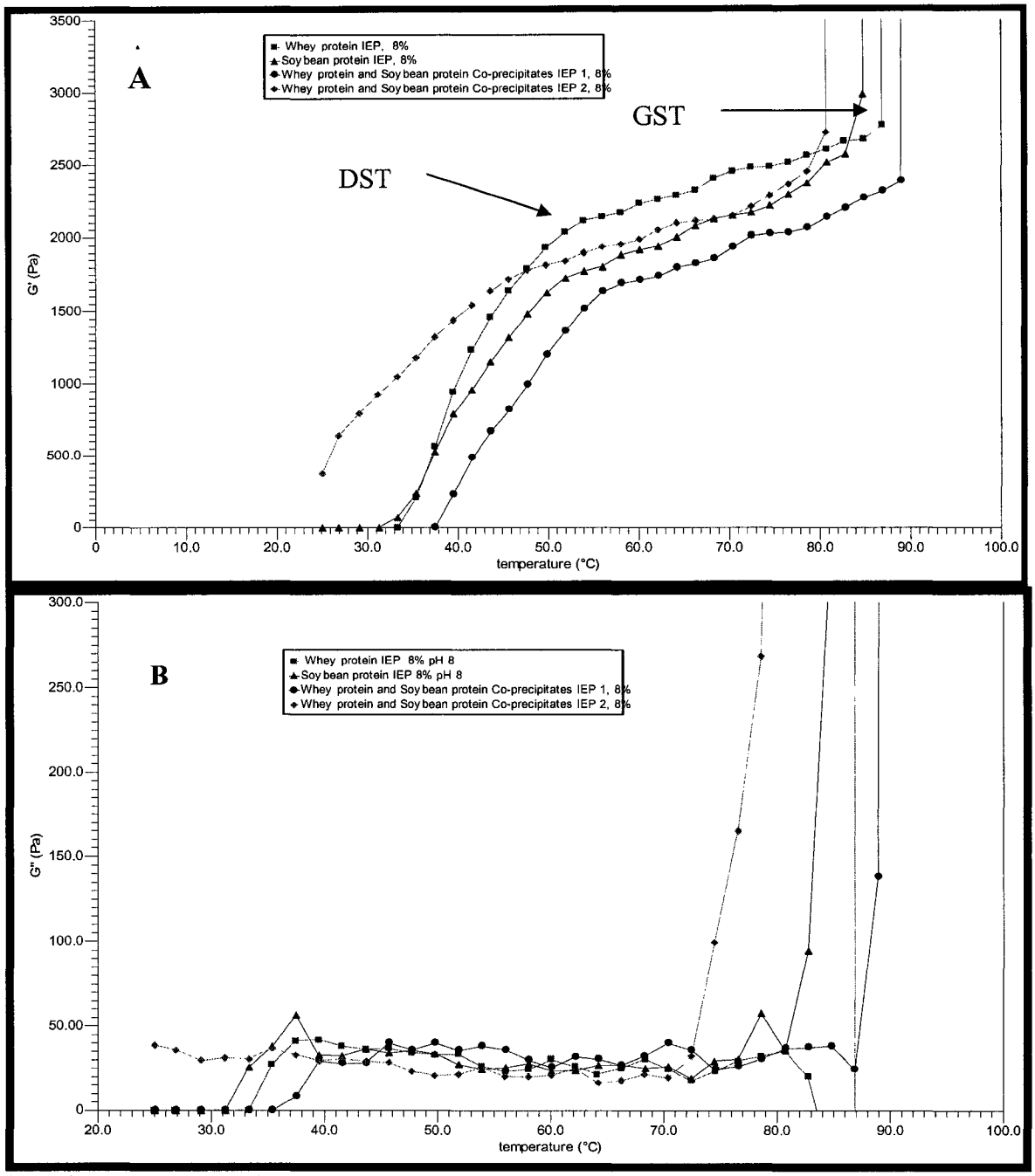


Figure 4.16: Effect of concentration on (A) Gel Strength and (B) Water Holding Capacity (WHC) for protein co-precipitates gel MP: and ME: NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling.

4.5.3 Rheological Properties of Protein Co-precipitates

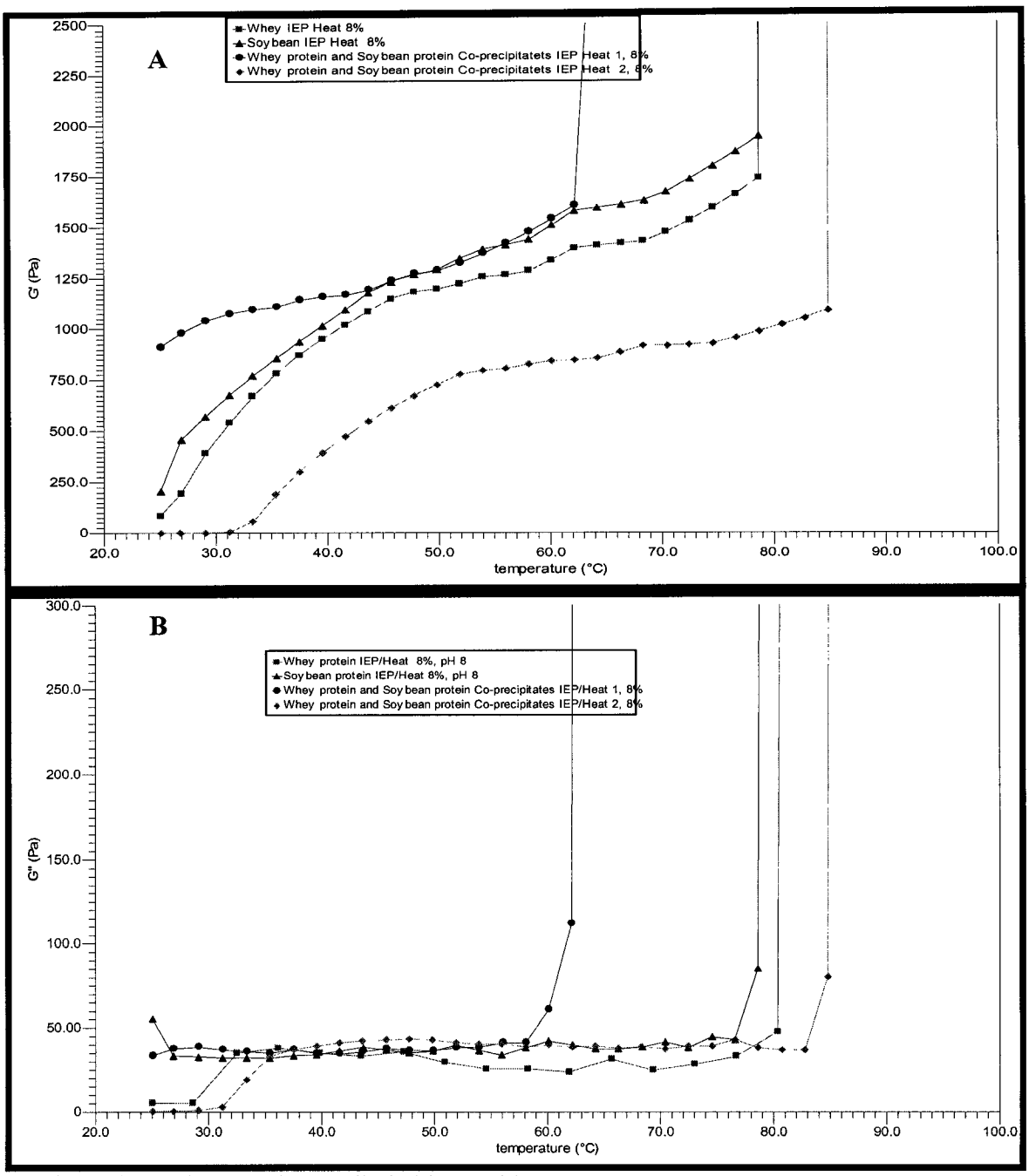
4.5.3.1 Effect of Temperature on Rheological Properties

Figures 4.17, 4.18 and 4.19 show the effect of heating temperature on the storage modulus G', loss modulus G" for whey protein precipitates, soybean protein precipitates and protein co-precipitates from MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling, for all protein precipitates the storage modulus G' was higher than the loss modulus G", indicating that the protein dispersions were more elastic than viscous. The elasticity increased with increasing temperature until denaturation start temperature DST, but after increasing the temperature above DST, then increased more gradually until the gelation start temperature GST was reached (4.17 A, 4.18 B, 4.19 A). No Change was observed in loss modulus during heating of the co-precipitates (Figure 4.17 B, 4.18 B and 4.19 B). This suggests an increase in the elasticity of the gel without change in viscosity during heating. During the heating (Figure 4.17 A), whey protein precipitates:NaOH/IEP-Cooling and soybean protein precipitates:NaOH/IEP-Cooling showed an initial G' increase at 32°C (0 Pa), while protein co-precipitates MP:NaOH/IEP-Cooling showed initial G' increase at 38°C (0 Pa). However for the protein co-precipitate ME:NaOH/IEP-Cooling, the initial G' increase was at 24°C (G' 350 Pa), suggesting that the protein was somewhat denatured during the co-precipitation preparation. The results in figure 4.19 suggest that the following co-precipitates whey protein precipitate, soybean protein precipitate and MP:NaOH/IEP-Heating-Cooling, also were denatured during the preparation.



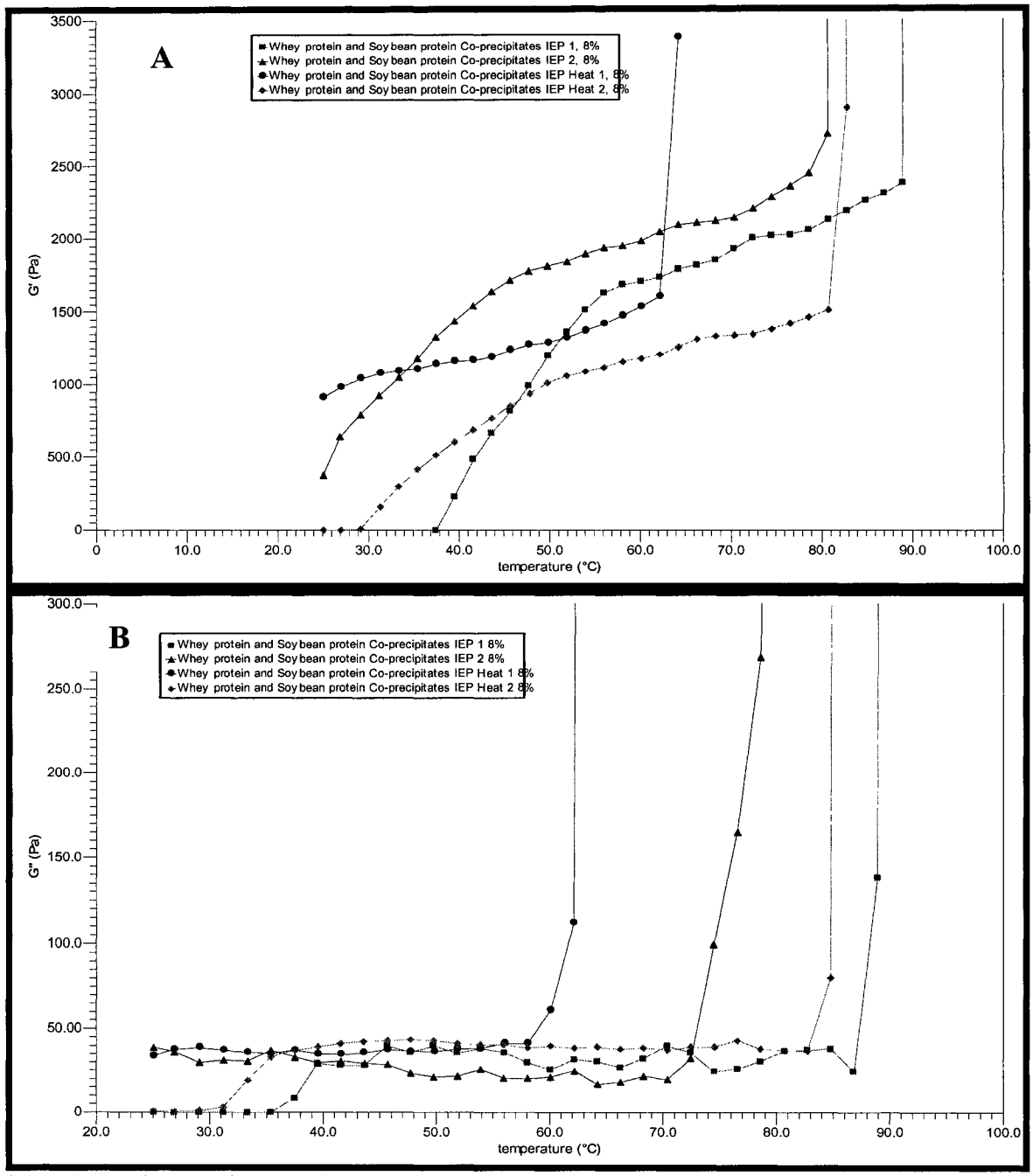
*IEP: (NaOH/IEP-Cooling); (1): (MP); (2): ME.

Figure 4.17: Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for soybean protein precipitate, whey protein precipitate and protein co-precipitates MP:and ME:NaOH/IEP-Cooling.



*IEP/Heat: (NaOH/IEP-Heating-Cooling); (1): MP; (2): ME.

Figure 4.18: Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for soybean protein precipitate, whey protein precipitate and protein co-precipitates MP:and ME:NaOH/IEP-Heating-Cooling.



*IEP : (NaOH/IEP-Cooling); IEP/Heat: (NaOH/IEP-Heating-Cooling); (1): MP; (2): ME.

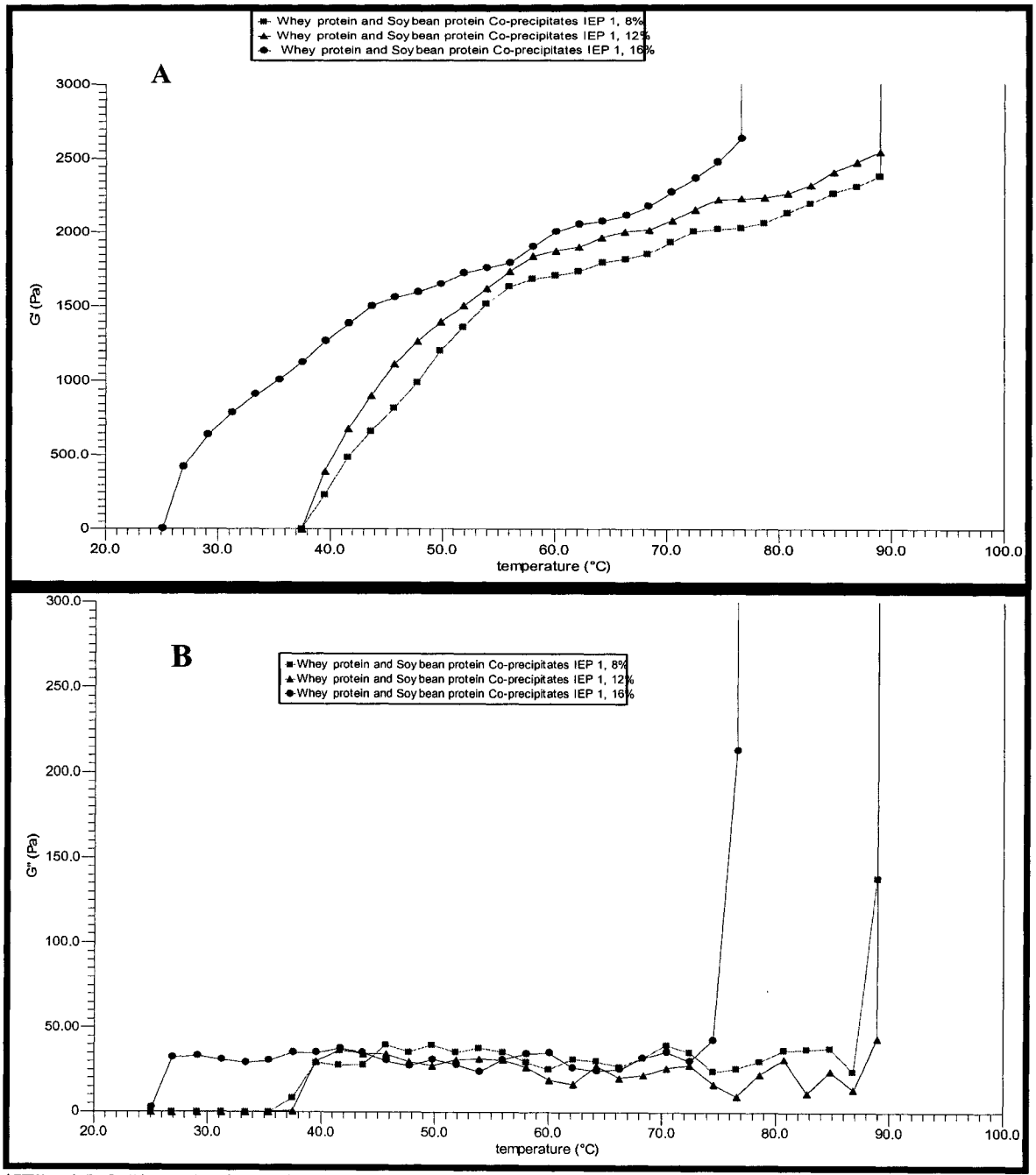
Figure 4.19: Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates MP:and ME:NaOH/IEP-Cooling and MP:and ME:NaOH/IEP-Heating-Cooling.

4.5.3.2 Effect of Concentration Rheological Properties

Figures 4.20, 4.21, 4.22 and 4.23 show the effect of protein concentration on the storage modulus G', loss modulus G" for protein co-precipitates MP: and ME:NaOH/IEP-Cooling and MP: and ME:NaOH/IEP-Heating-Cooling. The effect of protein concentration was much greater on storage modulus G' than on loss modulus G", suggesting that the gels were predominantly elastic gels. At the onset of the heating, protein co-precipitates 16% ME:NaOH/IEP-Heating-Cooling, 8% ME:NaOH/IEP-Cooling and 8%, 12%, 16% MP:NaOH/IEP-Heating-Cooling showed a storage modulus G'~80, 350, 600, 875, and 1400 Pa, and loss modulus G"~15, 40, 30, 0 and 170 Pa, respectively. This suggests that these protein co-precipitates were denatured to various extents during the co-precipitation preparation.

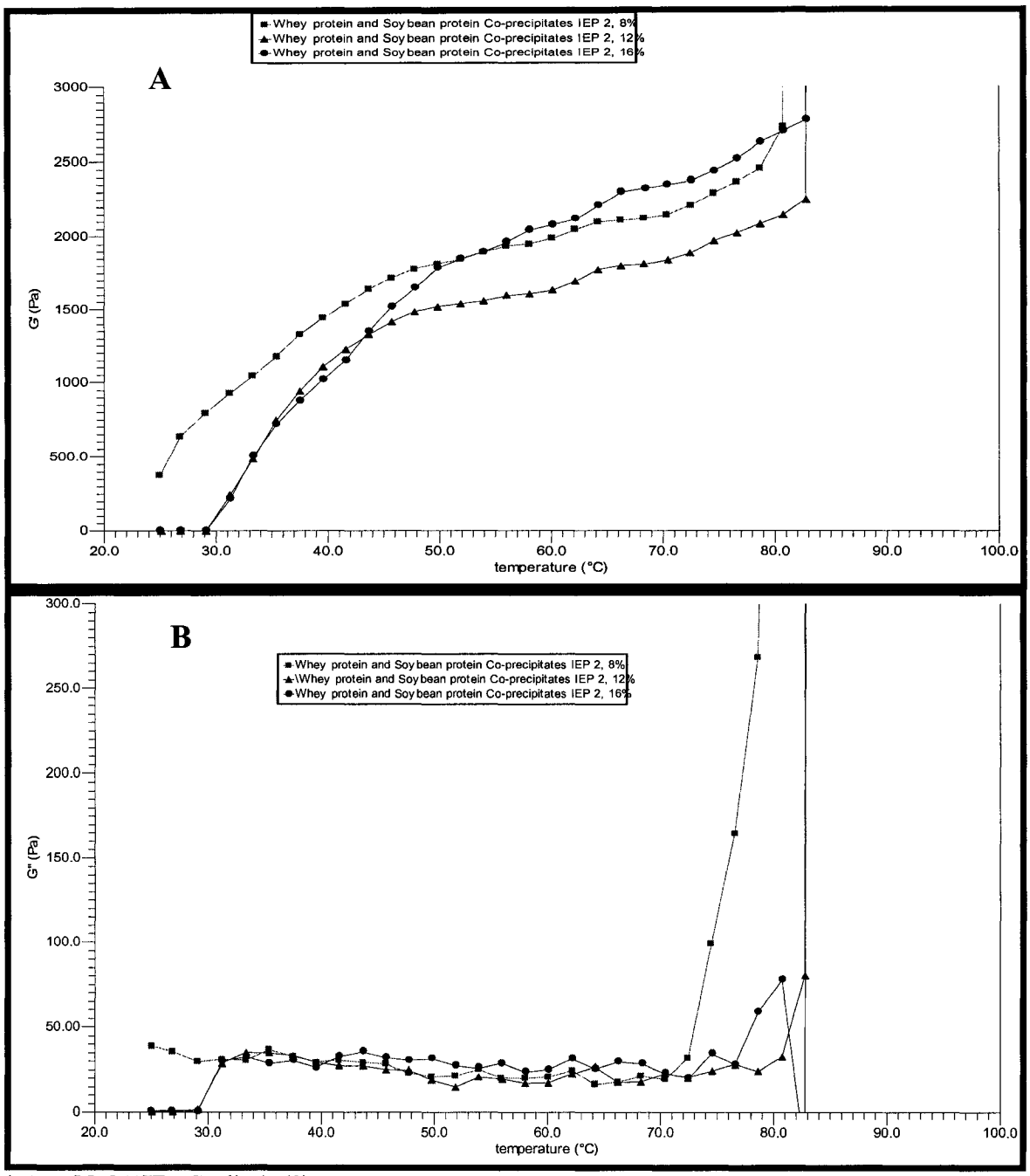
The NaOH/IEP-Cooling co-precipitate showed a marked increase in G' values with increasing concentration; there was a little change G" during heating of protein co-precipitates MP: and ME:NaOH/IEP-Cooling similar to each others as shown in Figure 4.20 B, 4.21 B. This suggests an increase in the elasticity of the gels and without change in viscosity of the samples.

Dispersion from 16% MP:NaOH/IEP-Heating-Cooling showed higher G" followed by dispersions from 12% and 8%, respectively (Figure 4.22 B). This suggests an increase in both gel elasticity and viscosity with increasing concentration. For the G" for 16% MP:NaOH/IEP-Cooling showed a decrease with heating temperature, with a slight decrease at 8% and 12% protein concentration. This suggests a decrease in gel viscosity with increasing elasticity, with increasing concentration.



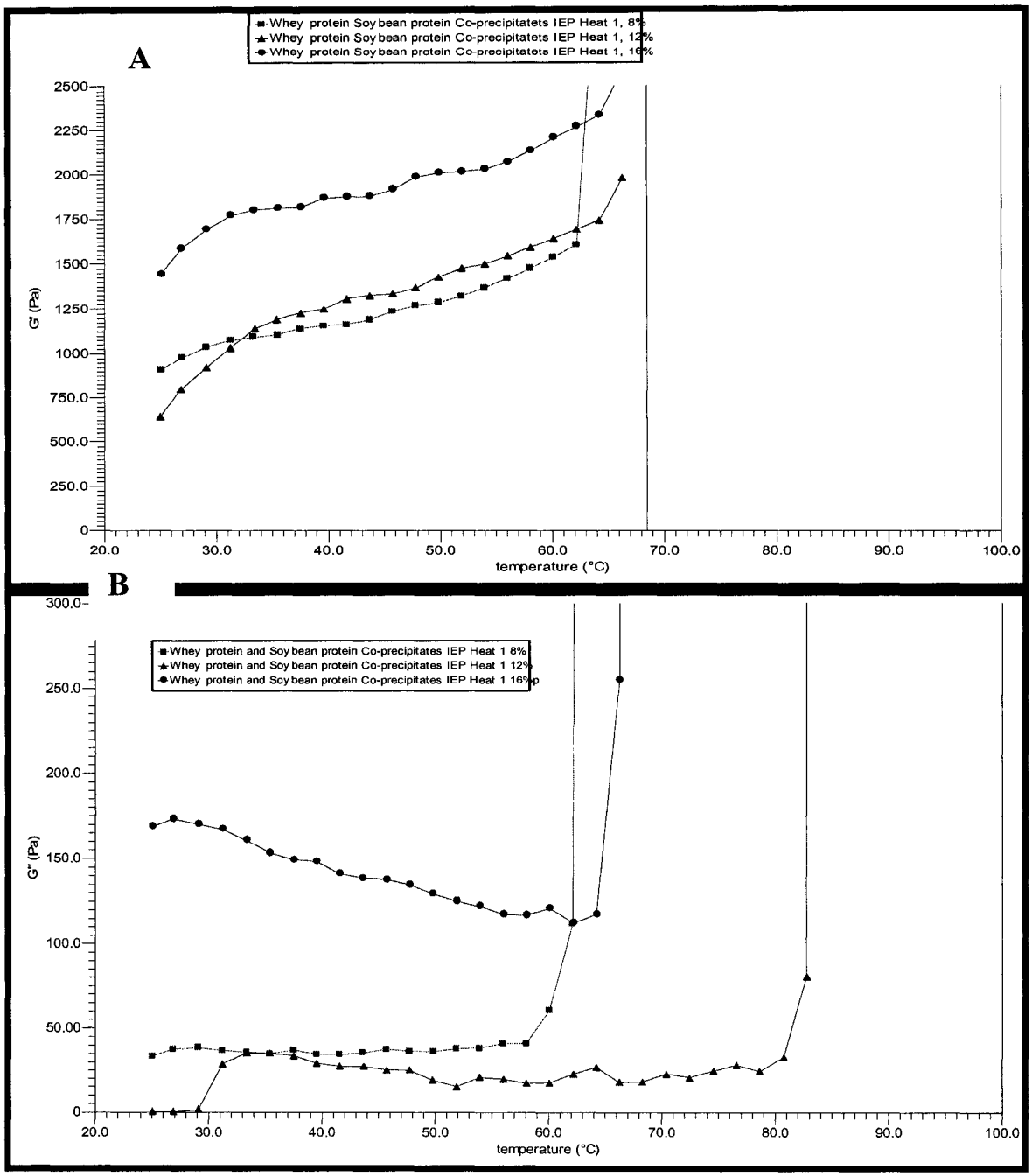
*IEP : (NaOH/IEP-Cooling); (1): MP.

Figure 4.20: Effect of concentration on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates MP:NaOH/IEP-Cooling.



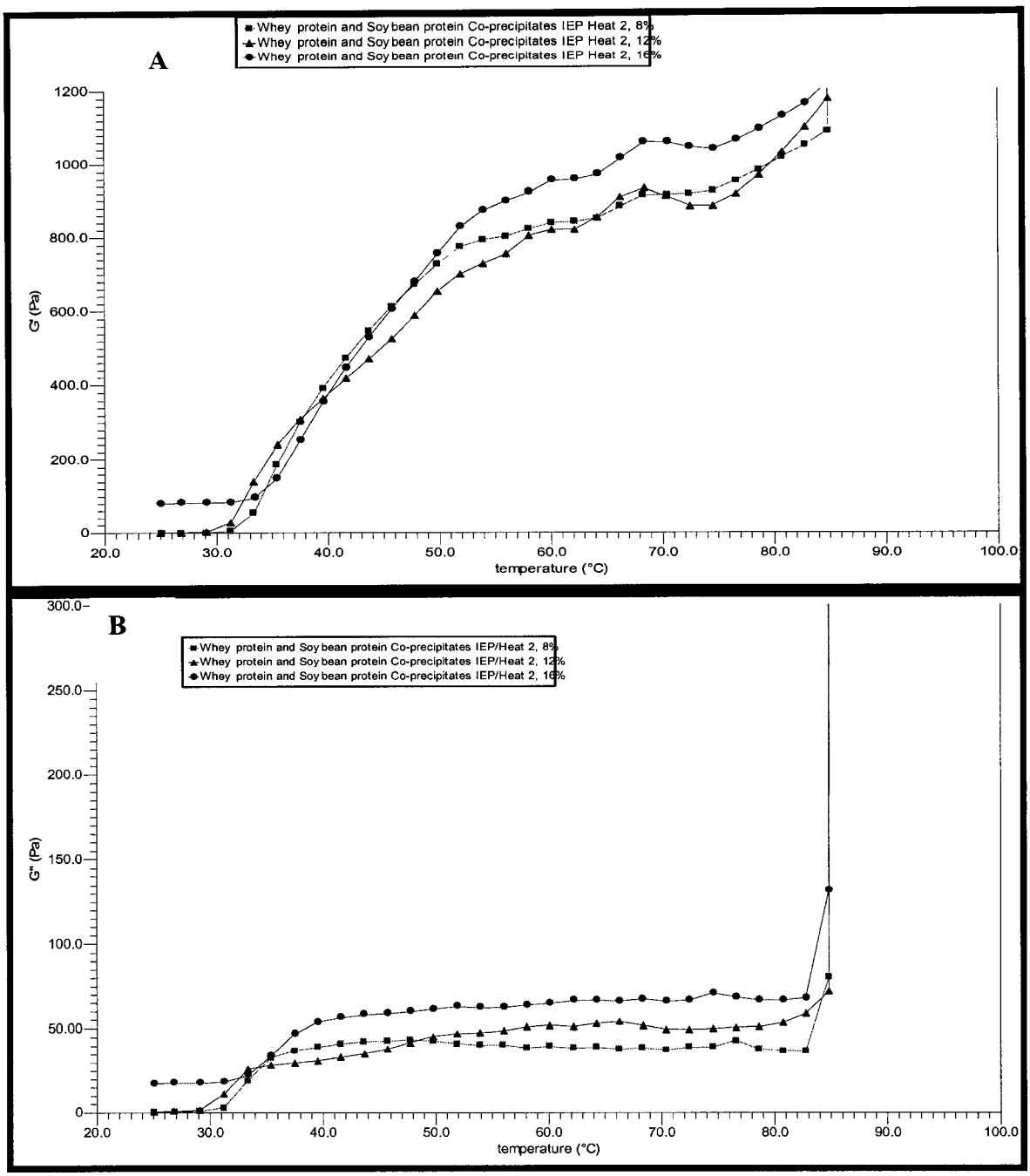
*IEP : (NaOH/IEP-Cooling); (2): ME.

Figure 4.21: Effect of concentration on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates ME:NaOH/IEP-Cooling.



*IEP/Heat: (NaOH/IEP-Heating-Cooling); (1): (MP).

Figure 4.22: Effect of concentration on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates MP:NaOH/IEP-Heating-Cooling .



*IEP/Heat: (NaOH/IEP-Heating-Cooling); (2): ME.

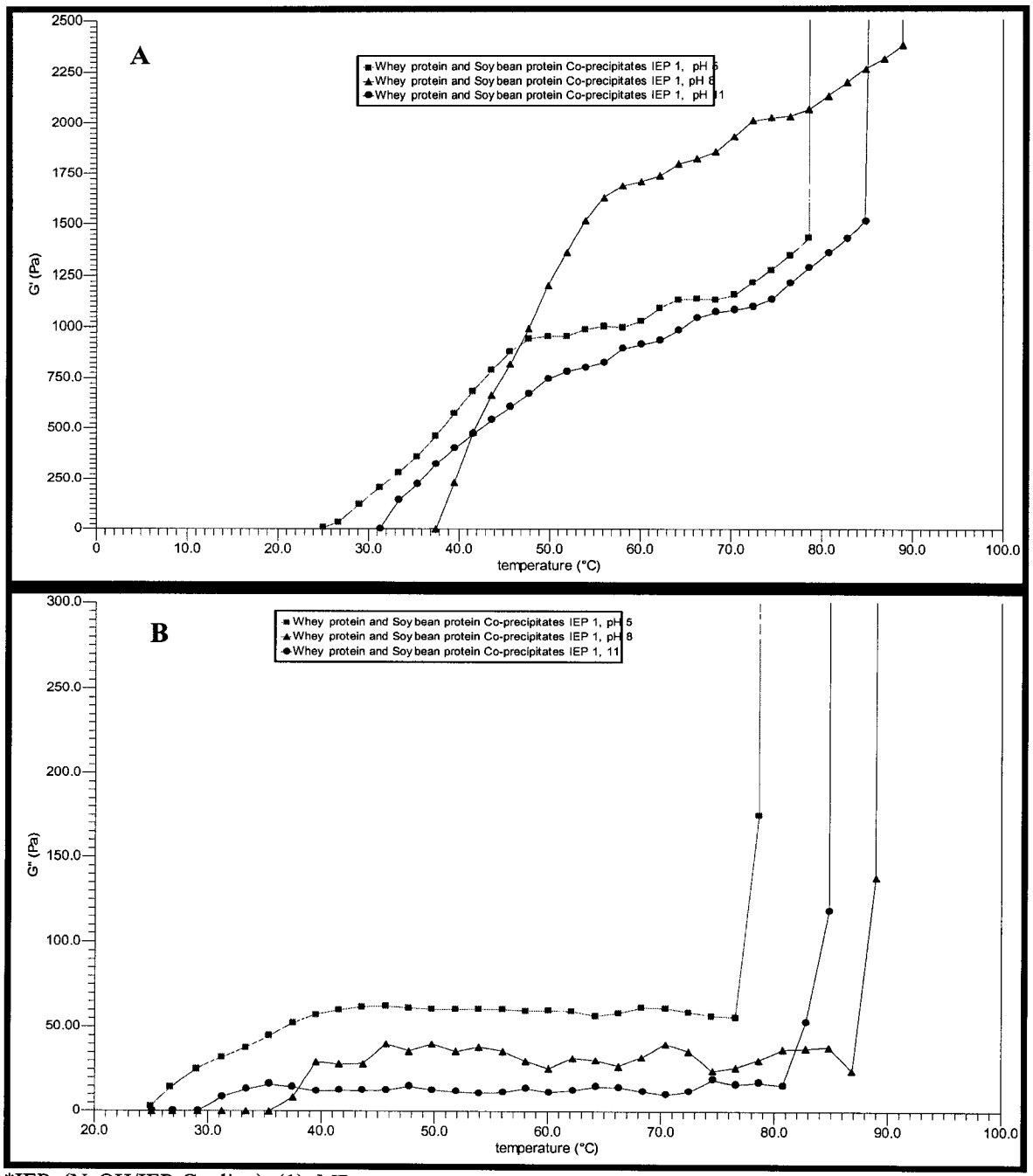
Figure 4.23: Effect of concentration on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates ME:NaOH/IEP-Heating-Cooling.

4.5.3.3 Effect of pH on Rheological Properties

Figures 4.24, 4.25, 4.26 and 4.27 show the effect of pH on the storage modulus G' and loss modulus G" for protein co-precipitates from MP: and ME:NaOH/IEP-Cooling. The storage modulus increased above 0 Pa with temperature above 25°C for pH 5, 31°C at pH 11 and 38°C at pH 8. The co-precipitate MP:NaOH/IEP-Cooling showed highest G" at pH 5 than at pH 8 and 11. This suggests an increase in both gel elasticity and viscosity with decreasing pH (Figure 4.24 B).

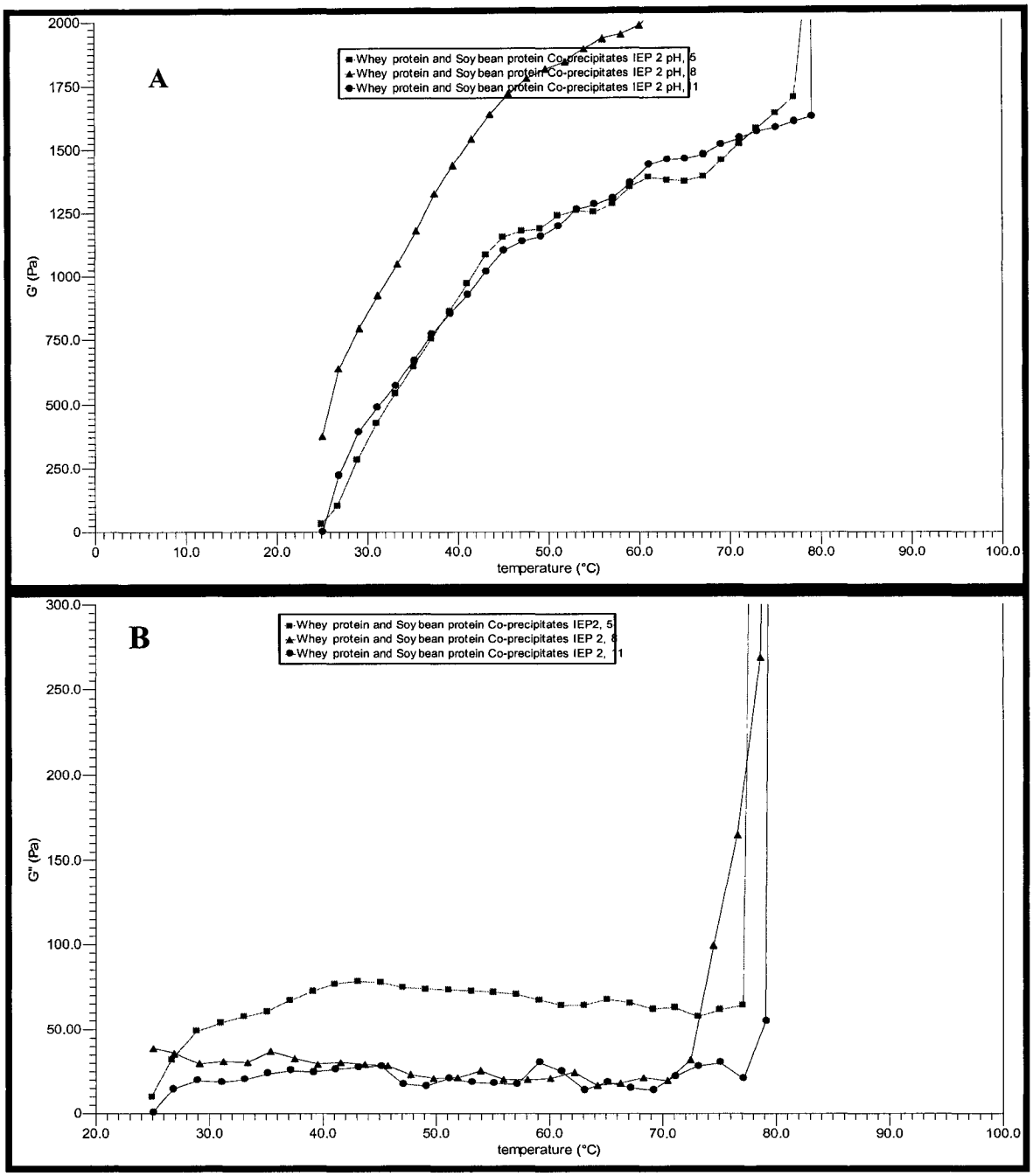
At pH 5 and 11 the protein co-precipitates ME:NaOH/IEP-Cooling showed an increase in G' above 0 Pa at 25°C; at onset of heating, the protein co-precipitate pH 8 showed G'~350 Pa. This suggests that the co-precipitates were denatured during the preparation of the co-precipitate. There was little change in the G" for protein co-precipitates ME:NaOH/IEP-Cooling at pH 8 and 11 (Figure 4.25 B).

For the co-precipitates of MP and ME:NaOH/IEP-Heating-Cooling (Figure 4.26 and 4.27), G' increased above 0 Pa with increasing temperature above 25°C for pH 5 and pH 11. No change was observed in loss modulus G" during heating for protein co-precipitates ME:NaOH/IEP-Heating-Cooling with increasing pH. This suggests an increase in the elasticity of the gel and without change in viscosity, for all pH values studied. The loss modulus for MP:NaOH/IEP-Heating-Cooling at pH 5 decreased with heating temperature, at all pH; the decrease was greater at pH 5. This suggests a decrease in gel viscosity with increasing elasticity with decreasing pH. The co-precipitates MP:NaOH/IEP-Heating-Cooling at pH 5 showed higher G" than at pH 8 and 11 Figure 2.26 B. This suggests increase in both gel elasticity and viscosity, with decreasing pH.



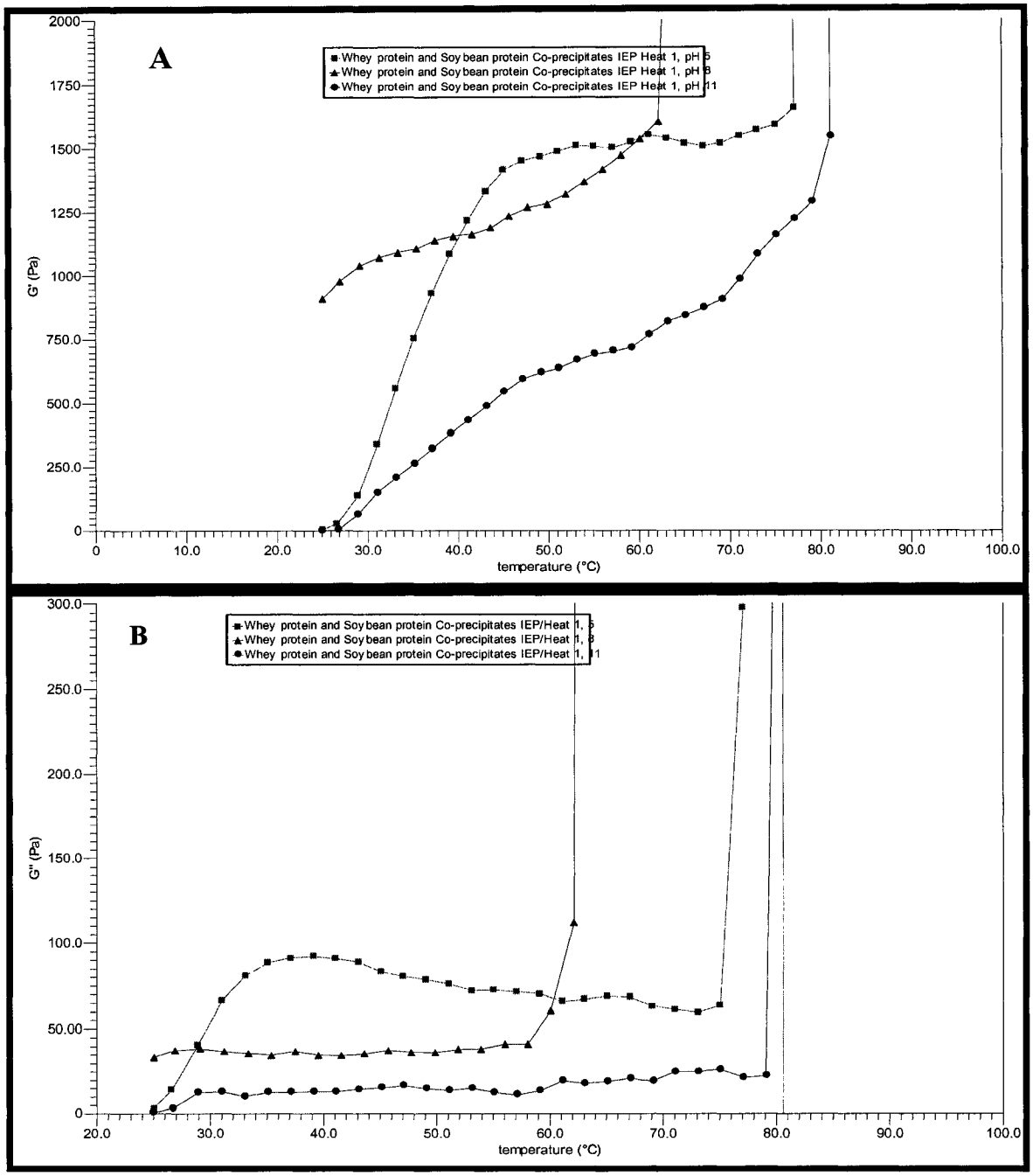
*IEP: (NaOH/IEP-Cooling); (1): MP.

Figure 4.24: Effect of pH on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates from MP:NaOH/IEP-Cooling.



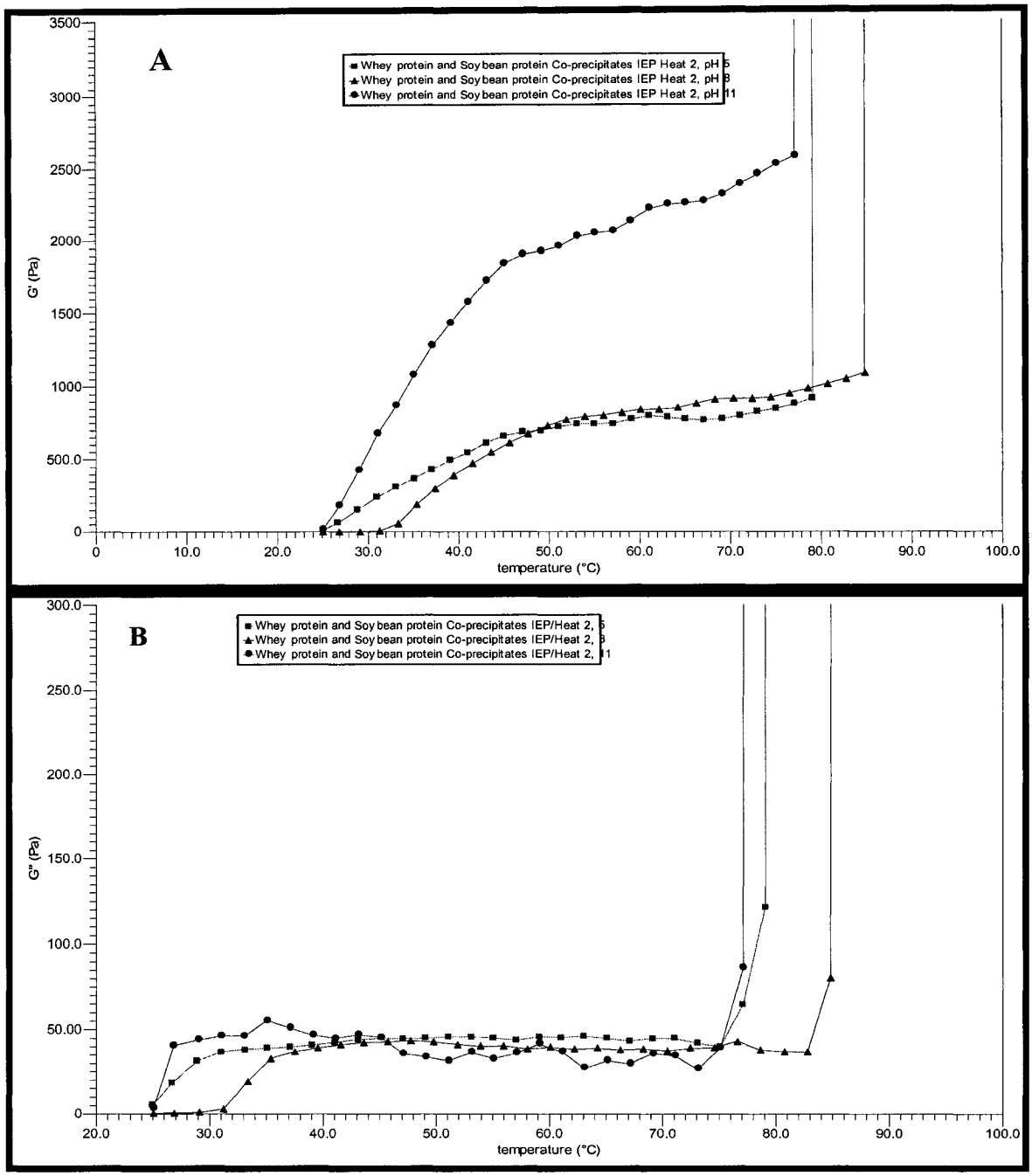
*IEP: (NaOH/IEP-Cooling); (2): ME.

Figure 4.25: Effect of pH on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates ME:NaOH/IEP-Cooling at 8%.



*IEP/Heat: (NaOH/IEP-Heating-Cooling); (1): MP.

Figure 4.26: Effect of pH on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature (G') and (B) changes in loss modulus (G'') with temperature for protein co-precipitates MP:NaOH/IEP-Heating-Cooling.



*IEP/Heat : (NaOH/IEP-Heating-Cooling); 2: ME.

Figure 4.27: Effect of pH on Dynamic viscoelastic modulus, (A) changes in storage modulus (G') with temperature and (B) changes in loss modulus (G") with temperature for protein co-precipitates ME:NaOH/IEP-Heating-Cooling.

CHAPTER 5

GENERAL CONCLUSIONS

This study investigated the conditions for preparing protein co-precipitates from whey powder protein and defatted soybean flour. The extraction conditions used were NaOH, NaOH-Papain and NaOH-NaCl; the conditions for protein co-precipitation were IEP-Cooling and IEP-Heating-Cooling and Cooling. The effects of the following factors on yield were also investigated: chelating agents, pH and temperature of precipitation.

The conditions, which resulted in the highest yield, were a combination of extraction conditions as well as a combination of co-precipitation techniques. Yield was affected by factors such as pH, chelating agents and temperature of precipitation. The highest yield of protein was obtained by use of the following extraction/co-precipitation conditions: extraction temperature (40°C), whey/soybean ratio (5:10), pH of extraction (11), pH of precipitation (4.5) and temperature of precipitation (95°C).

In general, all protein co-precipitates, which were investigated, showed protein components and their subunits, identified to those whey and soybean proteins. Native-PAGE showed that the interaction between the proteins resulted in at least two new bands, which did not correspond to either whey or soybean proteins. However, with SDS-PAGE the two bands gave subunits identical to those of whey and soybean proteins. This suggests that the protein-protein interactions, which occurred during formation of the protein co-precipitates, were reversible.

Gelation and rheological properties of the following protein co-precipitates were investigated: MP:NaOH/IEP-Cooling, ME:NaOH/IEP-Cooling, MP:NaOH/IEP-Heating-Cooling and ME:NaOH/IEP-Heating-Cooling. In general, protein gels obtained from

MP:NaOH/IEP-Cooling co-precipitates at 16% protein concentration showed superior gel strength and water holding capacity than those gels of either whey protein or soybean protein alone. Highest gel strength and water holding capacity was obtained with 16% protein concentration.

This study demonstrated a protein co-precipitate MP:NaOH/IEP-Cooling prepared from whey powder and defatted soybean flour was obtained in relatively high yield (27%) and had superior geleing properties; however, highest yield (45%) was obtained with MP: and ME:NaOH/IEP-Heating-Cooling. These two co-precipitates have potentially for further investigation towards their applications as food protein ingredients.

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