AN ABSTRACT OF THE THESIS OF

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Title: Characterization of Yellow Pea (*Pisum sativum* L.
          Miranda) Proteins and the Proteinate Functional
          Properties

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    Response surface methodology was used to study yield of

protein extractions, nitrogen content and solubility, least

gelation concentration, fat adsorption, and emulsifying

power of protein from yellow peas. Independent variables

were temperature °C(T) and pH(P), with acid (pH=4.0, AP),

$MgSO_4$ anhydrous (MAP) or $CaCl_2$ $2H_2O$ (CAP) as coagulants.

Amino acid composition, calcium content, and molecular

weight patterns were also evaluated. Amino acid composition

showed similar ratios of hydrophilic to hydrophobic residues

(60:40) with all proteinates, with AP containing the lowest

amount. Molecular weight patterns were no different among

the three proteinates, with MWs from 17-84 kD per subunit;

the products showed S-S bonds, mostly at subunit with a

MW=63 kD. CAP contained 3.04% Ca, compared to only 0.03% for

both AP and MAP.

    All proteinates contained similar amounts of N

(12.2-12.7%). Although N was similar, the proteinates had

different solubilities. AP had the highest solubility (9% at

pH 5.0 to 81% at pH 7.0), while the other two were 4% at pH

5.0 to 17% at pH 7.0. Yield of AP was significantly increased with the increase of T and P, CAP was significantly increased with the increase of P only, while MAP was not affected by either factor. There were maximum yields of 20.0, 16.8, and 19.0% for AP, MAP, and CAP, respectively, which recovered 62-74% of protein in the yellow pea flour.

Minimum concentration to form gel (LGC) was 18% for AP, and 15% for MAP or CAP. Maximum fat adsorption of AP was 534% (T=30.0^{0}C, P=8.9), of MAP and CAP were 522% (T=18.3^{0}C, P=10.0) and 510% (T=13.9^{0}C, P=10.3), respectively. Emulsion capacities were 62, 66, and 58 mL/g proteinate for AP, MAP and CAP, respectively, with AP as the most stable.

Characterization of Yellow Pea (*Pisum sativum* L. Miranda)

Proteins and the Proteinate Functional Properties

by

Uken Sukaeni Sanusi Soetrisno

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Typed by Uken Sukaeni Sanusi Soetrisno

"kesabaran mamah serta kerja keras bapak"
selalu meringankan langkah dan memacu semangat

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CHAPTER 1

INTRODUCTION

An increase in health and nutrition awareness in modern
society has meant that people choose more plant protein
sources that are low in fat, cholesterol, and sodium
(Anonymous, 1991a; Slavin, 1991). While in other parts of
the world where malnutrition is still rampant, food high in
nutrient density, especially affordable food protein, is
needed.

These concerns encourage more research in improving the
quality of conventional protein sources and developing new
unconventional ones. Soybean proteins are increasingly used
by the food industry to provide nutritional quality and a
variety of functional properties (Kinsella, 1979; Lambert et
al., 1989). Vegetable proteins as ingredients impart
desirable structure, texture, flavor and color
characteristics to formulated food products. Such products
are either meat analogs, meat, seafood or cheese extenders,
or are incorporated into baked and confectionery goods. This
increased utilization of legume protein by the food
industry, especially soybean protein (Anonymous, 1991b;

Duxbury, 1991; Kinsella, 1979), has accelerated research in the utilization of other legume or seed proteins in foods (Kim et al., 1990; Gebre-Egziabher and Sumner, 1983; Thompson, 1977; Rhee et al., 1972; Kantha and Erdman, 1984).

It has been widely reported elsewhere that chemical properties, such as nitrogen or protein content, solubility, and amino acid composition will greatly affect nutritional and functional properties of vegetable protein (Elizalde et al., 1991; Kinsella, 1979; Okezie and Bello, 1988). An expanded knowledge of protein structure, molecular size, and varietal differences of legumes or seeds will help food scientists to understand and manipulate their protein properties in food product development (Leterme et al., 1990; Kim et al., 1990; Wang and Damodaran, 1990; Wang and Damodaran, 1991; Cumming et al., 1973). Examination of protein characteristics will expand the choice of protein sources, give industry greater flexibility, decrease the pressure on the supply and availability of soybean, and give the consumer cheaper and more varied food.

A variety of yellow pea (*Pisum sativum* L. Miranda) has been successfully grown and yields a nutrient quality comparable to soybean when used in animal rations (England et al., 1986; Savage et al., 1986). In Oregon, approximately 15 million pounds are produced annually and sold for human consumption at relatively low prices (Carnes, 1988). If the protein in this yellow pea could be characterized and

developed into a viable product, it would be of economic benefit to the farmer.

The objective of this study was to investigate the yields, chemical characteristics, and selected functional properties of the yellow pea protein extracted by acid and salt coagulation. Central composite rotatable design was used to predict the extraction methods that will produce optimum yields and chemical and functional qualities.

CHAPTER 2

REVIEW OF THE LITERATURE

A major world concern in fulfilling the food need is to supply the protein required by the whole population now and in the future. Legumes such as peas, beans, and lentils are high in protein content and have been used as an inexpensive protein source in the diets where animal proteins are either unaffordable or are considered detrimental to the health and nutrition conscious population (Anonymous, 1991a). Oil seeds such as cotton seed, canola seed, and sunflower seed (Tzeng et al., 1988; Paulson and Tung, 1988) have also been investigated as a source for human food protein.

Legumes and seeds as protein sources are used as flours in products such as baby formula or supplemental diet for preschool children (Akinyele et al., 1988; Ulloa et al., 1988), baked products (Mustafa et al, 1986; Guy, 1984), pastas (Bahnassey et al., 1986; Molina et al., 1982) and extruded products (Aguilera and Kosikowski, 1976; Ringe and Love, 1988; Likimani et al., 1991). Since the flour form is needed in larger amounts to reach the same protein level than if using concentrate or isolate form, there have been attempts to extract protein from legumes and seeds. This extracted protein generally has a high protein concentration and provides nutritional quality due to lower

antinutritional factors with minimal off odor and color.

The concentrate and isolate forms are mostly used widely as an ingredient due to their functional properties (Kinsella, 1979) in food systems. Since every storage protein of seeds has its unique characteristics, these functional properties can be optimized by knowing the chemical and physicochemical characteristics of these proteins. Characterization is the initial step for modification and manipulation according to the purpose and need.

Uses of Vegetable Proteins

Soybean and other legume proteins have been used widely in replacing parts of wheat flour in bread and cookies. Sathe et al. (1981) used Northern bean flour or bean protein concentrates in bread mixtures. Results indicated that addition of up to 10% protein concentrate gave better dough and bread baking quality than that with bean flour, due to higher water absorption and less mixing time. Thompson (1977) evaluated the fortification of wheat flour bread with mungbean flour and its protein isolate. Addition of 10% isolate which gave 20.5% protein level still produced acceptable bread. To reach the same protein level, mungbean flour had to be added at 40% and produced unacceptable bread. Addition of stearoyl-2-lactylate could improve loaf

volume; however, texture and color were still inferior to the one with isolate.

Yellow pea, lentil, and faba bean protein isolate from both germinated and ungerminated seeds has been used to replace 3, 5, or 8% wheat flour in bread making (Hsu et al., 1982). All products were acceptable, except the ones with 8% protein isolate which had lower loaf volume, unsatisfactory crumb grain, and had pale crust color. Bread flour mixed with 12% soy flour and 1-3% whey solid (Guy, 1984) was an acceptable product and had 2 weeks longer shelf life after incorporation of 0.5% sodium steroyl lactylate in the mixture. Fortified bread and cookies with cowpea flour or its protein isolates at 10 or 20%, respectively, gave acceptable products, though 20% isolate gave a high spreading ratio (Mustafa et al., 1986).

Lorimer et al. (1991) studied the effect of globular proteins from navy bean (5 and 10%) substitution on wheat flour dough. The results showed that both high protein fraction bean flour and globular proteins increased absorption and arrival time in farinograph measures, but decreased stability. Combined with scanning electron micrograph evaluation and SH-SS interchange measurement, there was still not enough evidence to conclude that bean flour or globular protein disrupted the S-S interchange.

Defatted soybean and soy isolate have been added to wheat flour tortilla up to 15.5% protein content (Gonzalez-

Agramon and Serna-Saldivar, 1988). The protein efficiency
ratio was increased and the products had higher calories
than the control. Fortification caused increased water
absorption and decreased dough elasticity, but had no
adverse effect on product quality.

Spaghetti has also been fortified with edible roasted
and unroasted legumes and their protein concentrates, such
as navy bean, pinto bean, and lentils (Bahnassey et al.,
1986). The fortified products had a better balance of
lysine, sulfur containing amino acids, and minerals than the
controls. Addition of up to 10% legumes decreased yellow
color but was still acceptable. Pasta made of semolina-corn
mixes was fortified with 8% soy flour or 0.3% L-lysine
(Molina et al., 1982). Resistance to disintegration and
sensory evaluation indicated that quality decreased
significantly when semolina in the blend was lower than 40%
and 32%, respectively.

Extruded rice flour fortified with soy isolate (Noguchi
et al., 1981) had lower water absorption capacity, but less
protein insolubilization compared to nonfortified
extrusions. This was likely because of a sparing effect
between rice flour and soy protein isolate when processed
simultaneously. When the protein isolate was extruded by
itself, it formed new noncovalent interactions and disulfide
bonds resulting in decreased protein solubility.
Densitograms of polyacrylamide gel electrophoresis (PAGE)

and scanning electron micrograph (SEM) revealed that the 7S subunit was responsible for more noncovalent interactions and disulfide bonding and formed fine strings in the starch matrix, resulting in decreased protein solubility, compared to the 11S fraction.

In cowpea-corn flour extruded mixes (Ringe and Love, 1988), there was increase in lysine loss with increased storage temperature and time for all water activity levels. Losses were detected before the non-enzymatic brown pigment could be measured analytically. Nutrient composition of dry extruded cowpea products was studied (Akinyele et al., 1988) in an infant formula. Blended cowpea with rice or corn or both, with addition of banana to form the baby formula, had an average energy content of 440 kcal/100 g with a protein content of 20%. Trypsin inhibitor activity was reduced by 63-84%, and oligosaccharide content was low.

Chickpea protein concentrate has been studied in development of special infant formula (Ulloa et al., 1988) with and without methionine supplementation. The formula had higher Relative Net Protein Ratio (83.6%) compared to that of protein concentrate itself. This indicated no apparent change in protein quality during processing. In fact, extrusion cooking may improve nutritional and functional quality of the protein (Camire, 1991) through modification of the extrusion conditions. Antinutritional factors and undesirable compounds can be reduced, while flavors and

odors are improved.

Navy bean protein concentrate was added to retail ground beef as a meat extender (Duszkiewicz-Reinhard et al., 1988). Addition of protein concentrate lowered the pH of the mixture and production of lactic acid resulted in lower pathogenic or spoilage aerobic plate count and coliforms. The fat of comminuted meat-type product or bologna was substituted with tofu or soy protein concentrate (Jeng et al., 1988). Bologna with tofu had higher emulsifying capacity, tougher texture, less moisture stability and lower fat and caloric level than control or soy concentrate-bologna. Soy concentrate-bologna had more beany flavor and was more tender.

Rice et al. (1989) evaluated the effects of soy protein blends added to beef patties. They reported that combinations of textured and powdered soy proteins increased yields and nutrient retention of beef patties. Such products have been used to provide better balance of nutrients for school lunch (Engineered, 1991; Duxbury, 1991).

Seafood was extended with soy protein concentrate, structured protein fiber, and textured vegetable protein (Beuchat and Jones, 1979). In a simple system of a liquid culture medium, there was enhanced growth of *V. parahaemolyticus* by treatments with protein extender. However, *V. parahaemolyticus* was retarded in the ground flounder, shrimp, and crab, which were extended with 5-25%

soy concentrate or structured protein fiber. Texturized vegetable protein promoted the rate of microbial growth when added to seafood at levels of 5 and 10%. These inhibitory and stimulatory effects were attributed to possible differences in types of competitive microflora that might be indigenous to the soy extenders and to the chemical composition and physical form.

Peanut protein isolates from 3 different varieties (Conkerton and Ory, 1976) were used as protein supplementation in pine apple juice, as an acid type beverage, at 1% level. There was no differece on flavor, texture, or aroma, although the tubidity was slightly increased.

Methods of Protein Extraction

Many factors affect the extractability of protein, including particle size and quality of flour, solvent to flour ratio, pH and temperature during extraction, and ionic strength or addition of salts into extractant (Aguilera and Garcia, 1989; Kinsella, 1979). It is important to recover as much protein as possible during extraction to get maximum protein content in the concentrate or isolate products. A large number of reports (Appendix 1), have evaluated extraction techniques and processing conditions in a variety

of legumes and seeds. The studies analyzed yield and quality
of certain fractions of proteins, besides considering cost
and extraction time factor.

Effect of size and treatment of the particles on
extraction yield had been studied (Aguilera and Garcia,
1989). Smaller particle size increased protein yield, while
flaked or exploded material had higher diffusion
coefficients which gave higher yield. The extraction yield
of whole and dehulled yellow pea flour were studied (Sumner
et al., 1981). Results showed that dehulled pea flour gave
lower crude fiber and higher protein contents.

A positive relationship between protein curd yield with
protein recovery during solvent extraction (Wang et al.,
1983), but not with the protein content of the soybeans, has
been reported. Alkali extraction at pH 7.0-12.0, followed by
precipitation at pH 4.5, gave a protein yield of about 60-
92% (Ulloa et al., 1988) in chick peas. Yield was highest at
pH 12.0 but this required large amounts of NaOH that may
cause extreme changes in environment. The NaOH can also be
detrimental to protein quality.

Water and other solvent extractions (Appendix 1) at pH
7.0 could produce 80% or more protein yield. Combinations of
low ionic strengths and different pH levels of extractant
produced different protein fractions (Brooks and Morr, 1984;
Honig et al., 1984). Low temperature (0-10°C) during
extraction is important if the protein is to be evaluated

for its biological activity or its native storage protein. Most reported that extractions (Appendix 1) were done at room temperature (20-25°C).

Ultrafiltration, diafiltration, and ion exchange were applied following the extraction and precipitation of isolates (Appendix 1) to produce proteins that were free from or low in glucosinolates, phytates, fiber, tannins, or other impurities, and to produce a high yield with very high solubility indices (Kinsella, 1979). Co-extraction and co-ultrafiltration of diary and vegetable protein blends has been used (Nichols and Cheryan, 1982) to produce milk protein replacer that has good functional properties. Industrial membrane systems (Manak et al., 1980) have been used to produce protein isolates from soy, cotton seed, and peanut. The isolates contain >90% protein with good functional properties.

Temperature sensitive gels of poly iso propyl acrylamide has been used (Trank et al., 1989) to recover essentially all of the proteins, remove undesirable components, and improve nutritional and functional quality. Most extractions used flour to solvent ratios of 1:5 to 1:30 with 1 to 4 times repetitive extractions. It was revealed that 2 extractions were generally adequate (Rhee et al., 1972) since the third and fourth did not significantly increase yield. The optimum ratio reported for rape seed (Tzeng et al., 1988) or peanut (Rhee et al., 1972) was 1:20.

This made it easier to handle the total amount of solvent and reduce water volume.

Protein curd was produced from field peas (*Pisum sativum* var. Trapper) (Gebre-Egziabher and Sumner, 1983) using 1:5 flour to water ratio at pH 8.8-9.0. This was adjusted using 0.2% CaO. The pea milk was then coagulated using 2% CaSO4 until reaching 0.54% concentration. As expected the curd from $CaSO_4$ coagulant had a high ash content. Such coagulation would be a good source of Ca in countries where the milk supply is low or expensive.

The effects of drying method in preparing protein isolate from smooth seeded yellow pea (*Pisum sativum* var. Trapper) on chemical and functional properties was evaluated (Sumner et al., 1981). Alkaline extraction was employed followed by precipitation at the isoelectric point, producing isolate with 90% protein content. Drum drying, compared to freeze drying and spray drying, decreased the nitrogen solubility index and increased water absorption. This was likely due to protein denaturation during increased heating. Freeze dried and spray dried isolates had the highest emulsification and water absorption values. Spray drying produced the best foaming, color, and flavor properties. The spray dried protein content may have had less Maillard reaction or polyphenol oxidation.

Characterization of Proteins

Chemical characteristics and physical behavior of
protein extracts include amino acid composition, protein
conformation, charge distribution, molecular size,the extent
of inter- and intra-molecular bonding, and are influenced by
the environment such as temperature, pH and ionic strength.
Functional properties of proteins are the reflection of
those intrinsic physicochemical attributes and environment
conditions. High quality protein ingredients must fulfill
criteria, such as (Fenemma, 1985) contain high protein
concentration, provide nutritional quality, have minimal off
odor and color, are low amounts of toxic or antinutritional
factors, are readily available at low cost, have
compatibility with other ingredients under the processing
conditions, and have desirable functional properties.

Chemical characterization. Amino acid composition is
important to nutritional and functional quality of proteins.
Amino acids will contribute to the essential amino acid
content of the protein, which will determine its nutritional
value; and to total charge and disulfide bonds within the
subunit or between subunits to form polymers with flexible
or rigid structure.

Soybean globulins were quantitatively analyzed (Sato et
al., 1986) using densitometer of SDS-polyacrylamide slab gel
with Coomassie blue as the staining reagent. Conglycinin or

7S globulin contained 27.8% beta, 6.2% gamma, and 3.0%
basic-conglycinin and 2.9% trypsin inhibitor. Glycinin or
11S globulin was present at 36.5%, and other proteins were
23.6% of the total.

Size heterogeneity of cotton seed storage protein has
been characterized by SDS-PAGE gels (Marshall et al., 1984).
There were 6 fractions, with the larger subunits covalently
bonding, and smaller subunits of globulin fractions forming
aggregates of quaternary structure that were stabilized by
hydrophobic interactions. Molecular size of yellow pea
proteins has been investigated in a preliminary study by
this author using SDS-PAGE gel electrophoresis with 12%
acrylamide. The results indicated that this protein extract
consisted of 6 fractions with sharp bands and 7 fractions
with lighter bands with molecular weights ranging from 22 kD
to 81 kD. Water absorption of smaller molecular weight (15
kD) as result of bromelin treatment on the soy protein
(Mohri and Matsushita, 1984) increased by 2-2.5 times, due
to degradation of the 11S globulin. This degradation allows
for new associations through hydrophobic and disulfide
bonds. Water imbibing (Yao et al., 1988) of soy protein
decreased with increasing maturity, which correlated to the
increase in the ratio of 7S to 11S.

Isolation of 7S storage protein from cotton seed has
been done (Zarins et al., 1984) using electrofocusing in
polyacrylamide gels. The results showed that the 7S fraction

(molecular weights ranged 15-17 kD) consisted of basic units composed of 8 components differing in charge, while larger subunits consisted of 2 or more of these subunits. Protein modification in the form of molecule size reduction in yellow pea, lentils, and faba bean after germination was evaluated using SDS-PAGE and densitometric scanner (Hsu et al., 1982). There were increases in small subunit proteins after germination. Protein isolate from those germinated legumes had higher emulsion capacity, increased foam expansion, decreased foam stability, lower viscosity, formed soft and smooth curd; except protein isolate from germinated pea or lentils, which gave severe syneresis.

Physicochemical or Functional Characterization. Functional characterization of protein can be generalized as hydration, emulsification, textural, and rheological (Fenemma, 1985). Those characteristics can be measured through their nitrogen solubility, water absorption, viscosity, swelling, gelation, fat adsorption, foaming, whipping, adhesion, fiber/texture, aggregation, dough formation, and extrudability. Some of the measures will be discussed.

Nitrogen solubility is one aspect of hydration, which is the most important characteristic in evaluating protein quality since many functional properties of protein depend upon their capacity to go into solution initially. Solubility is affected by many factors, such as pH during

extraction or solubilization. Sathe et al. (1982) reported that lupin protein extracted at alkaline pH had greater dispersibility at pH >3.0. Highest solubility at pH 12.0 was reported on winged bean protein (Okezie and Bello, 1988); while the lowest solubility of protein from yellow pea, lentils, and faba bean were at pH 4.5-5.0 (Hsu et al., 1982) which were not affected by molecular size reduction due to germination.

Enzymatic hydrolysis increased the solubility of soy protein isolate (Kim et al., 1990) due to breakdown of the oligomeric structure of 7S globulin which caused alteration of charge properties. Highly denatured soy protein isolate had lower solubility due to increased free sulfhydryl content (Wagner and Anon, 1990), compared to isolate with a low degree of denaturation which had more native protein.

Gelation is another aspect of hydration and of textural and rheological properties of protein. It is defined as the formation of three dimensional intermolecular networks through hydrogen, hydrophobic, and disulfide bonds that entrap water and other ingredients. This entrapment contributes to texture and chewiness of the food products (Furukawa and Ohta, 1982; Utsumi and Kinsella, 1985). The important initial step in heat-induced gelation of globular proteins is heating of the protein solution above the denaturation temperature to expose the functional groups, so that the intermolecular network can be produced.

Additionally, high numbers of intermolecular disulfide bonds increased water holding capacity, and, as a result, increased gel hardness.

Wang and Damodaran (1990) studied the thermal gelation of globular protein of bovine serum albumin (BSA), soy isolate, 7S, 11S, and phaseolin. They reported that gel hardness or strength of globular protein gels is fundamentally related to the size and shape of the polypeptide in the gel network rather than to their chemical nature such as amino acid composition and distribution. Globular protein with MW<23 kD cannot form a self supporting gel network at any reasonable concentration.

The structural state of globular proteins from bovine serum albumin and soy protein in gels was studied (Wang and Damodaran, 1991). The results indicated that gelation of BSA involved transconformation of α-helix and aperiodic structures into β-sheet conformation. Conditions that decreased the formation of β-sheet structure decreased the gel strength. Soy proteins, which contain mainly β-sheet and aperiodic structures in their native state, only showed a reduction in β-sheet and an increase in aperiodic structure content in the gel state. It is hypothesized that in globular protein gels, intermolecular H-bonding between segments of β-sheets oriented either in parallel or in antiparallel configurations may act as junction zones in the gel network.

Soybean protein consists of 4 main components; 2S, 7S, 11S, and 15S. The 7S and 11S fractions are main storage proteins and account for about 70% of the total protein content. Saio and Watanabe (1978) studied the differences in gel formation of these 7S and 11S proteins. They reported that the 11S fraction formed harder gels with S-S bonds as the predominant bonding force and precipitated faster and formed larger aggregates. Gels made of 11S protein had higher water holding capacity, higher tensile volume, higher hardness, and expanded more on heating.

The study on proteolytic degradation of soy protein isolate (Kim et al., 1990) showed there was more than reduction of molecular size that increased thermal aggregation. 11S protein was reported as the most responsible for thermal aggregation, and especially because basic polypeptide precipitated almost quantitatively upon heating, whereas 7S globulin prevent their thermal aggregation through the formation of a soluble complex.

The effect of temperature, protein concentration, and proportion of glycinin to conglycinin on the mechanical parameters of soy protein gels were reported (Kang et al., 1991). The gels formed at higher heating temperature and protein concentration were firm, tough, and unfracturable. Elasticities were similar at all concentrations and lower when heated at higher temperature. Heating above 93^0C was necessary for formation of rigid gels. Glycinin to β-

conglycinin ratios affected the texture; low glycinin gels were more elastic than regular gels, thus indicating that β-conglycinin largely contributes to elasticity.

Yang and Taranto (1982) evaluated the textural properties of mozarella cheese analogs manufactured from soybean protein. The results showed that physical and functional properties of the final product, which were prepared from soy protein, gelatin, and gum arabic, were found to be similar to those of natural mozarella cheese. Stretchability of the product is related to the amount of soy protein. Addition of salts, such as calcium chloride, was found to improve the stretchability of the progel.

Emulsification properties include fat adsorption, foaming, and whipping abilities. The oil-in-water emulsion stabilized by protein was represented by a bilayer model (Elizalde et al., 1988). Protein diffused and reoriented at the oil-water interface, the hydrophilic groups oriented towards the water phase and the hydrophobic groups towards the oil. The thickness of the interfacial bilayer depended on the water and oil absorption capacity of the proteins and on the concentration of the protein. Protein-protein interactions were important to enhance cohesive forces between the proteins and hence the rigidity and mechanical strength of the bilayer film to prevent rupture and coalescence of oil globules.

Emulsion stability is enhanced by high protein and oil

concentration (Elizalde et al., 1991), and these factors are highly interrelated. Temperature variation, 37, 45, and 60^{0}C, had little effect on emulsion stability as measured by the energy activation. They also reported that emulsion stability depended primarily upon the WAC (water absorption activity) and OAC (oil absorption activity) of protein. Proteins with low WAC and WOAI (water-oil absorption index) are not suitable for formulation of stable emulsions, even if using high concentrations or formulation with high oil concentration.

Emulsion properties of yellow pea flour with different particle size were studied (Horvath et al., 1989). They reported that flour with higher protein content had greater emulsifying capacity. Fine particles had high oil absorption capacity and good emulsifying capacity and stability, while coarse particles had high WA capacities. All samples had high NSI and were considered to have very promising functional properties.

Emulsion capacity measurement for non meat proteins was discussed (Amundson and Sebranek, 1990). Addition of NaCl or added protein to water, as opposed to oil, decreased the end point of soy protein isolate emulsion. On the other hand, a low NaCl levels increased the end point of Na-caseinate emulsion, and the order of water or oil addition had no effect.

Textural property of texturized plant protein is very

important, as it needs to resemble meat texture. To produce a texturized product, plant protein must comply with certain minimum prerequisites. A protein ingredient to be extruded (Kazemzadeh et al., 1982), must have a minimum protein content of 50%, a nitrogen solubility index of 50-70, a maximum soluble carbohydrate content of 30%, and less than 1% fat. Defatted soy meal can be converted into a fibrous, porous product with meat-like texture by use of high temperature, high pressure, and short time extrusion (Stanley and deMan, 1978). A possible mechanism is the dissociation of protein in high heat and pressure into subunits followed by formation of high molecular aggregates through intermolecular amide bonds.

Protein level and temperature had a positive relationships with the shear force as a texture indicator (Kazemzadeh et al., 1986). According to mechanical and microscopic evaluations, high protein content is essential for well-developed texture in soybase mixtures, with the assumption that proteins contribute to the skeletal structure in which carbohydrates are dispersed. There was a pronounced increase in all of the measured parameters between 40 and 70% soy protein isolate content.

The possible role of crude fiber of soy flour in texture formation during nonextrusion texturization processing (Taranto et al., 1981) was studied. The results indicated that crude fiber may control the type of

alveolation developed and the type of cuticle morphology
exhibited in the products alveoli as a result of its plastic
response to deformation. It seems probable that the presence
of the soluble carbohydrate fraction is not a prerequisite
for the formation of the alveolate morphology. The
structural integrity of the final texturized product was
lowered as the amount of crude fiber present in the soy
flour mixture was increased from 5 to 12%.

CHAPTER 3

PROTEIN YIELDS AND CHARACTERISTICS FROM ACID AND SALT

COAGULATIONS OF YELLOW PEA (*Pisum sativum* L. Miranda) FLOUR

EXTRACTIONS

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PROTEIN YIELDS AND CHARACTERISTICS FROM ACID AND SALT COAGULATIONS ON YELLOW PEA (*Pisum sativum* L. Miranda) FLOUR EXTRACTIONS

Abstract

Acid proteinate (AP), magnesium proteinate (MAP), and calcium proteinate (CAP) extracted from yellow pea flour at different pH (P) and temperature ^{0}C (T) levels were analyzed. The yield (g/100 g flour) of AP (maximum 20.2%; T=32.1^{0}C, P=9.8) was significantly affected (P<0.10) by different combinations of temperature and pH during extraction, while yield of CAP was only affected (P<0.001) by pH (max=19.0%: T=27.0^{0}C, P=10.8), and MAP (max=16.8%) was not affected by either factor. There were no differences in nitrogen content (70.7% protein) for the three proteinates due to temperature and pH variables. Hydrophilic amino acid contents of AP, MAP, and CAP were 64.2, 61.8, and 62.1%, respectively. Cysteine and methionine content were the lowest for all proteinates. Gel electrophoresis of all proteinates showed similar molecular weight subunit patterns, ranging from 17 to 84 kD with disulfide-bond generally at MW of 63 to >100 kD.

Introduction

Increased utilization of legume protein by the food industry, especially soybean protein (Kinsella, 1979), has increased research on the utilization of legume or seed proteins in foods (Kim et al., 1990; Gebre-Egziabher and Sumner, 1983; Thompson, 1977; Rhee et al., 1972). Legume or seed protein is used as an ingredient, primarily to increase nutritional quality and provide a variety of functional properties, including desirable structure, texture, flavor, and color characteristics in formulated food products.

Knowledge of protein structure and its molecular size in different legumes or seed, or different varieties, will bring about an understanding of the protein properties. This will permit manipulation of these properties for food product development (Leterme et al., 1990; Kim et al., 1990; Wang and Damodaran, 1990; Cumming et al., 1973). Nutritional and functional qualities of a protein are largely determined by its amino acid content and nitrogen solubility (Kinsella, 1979).

A new variety of yellow pea (*Pisum sativum* L. Miranda) has been successfully grown and yields a nutrient quality comparable to those of soybean when used in animal rations (England et al., 1986; Savage et al., 1986). In Oregon, approximately 15 million pounds are produced annually and sold for human consumption at relatively low price (Carnes,

1988). If the protein in these yellow peas could be characterized and developed into a viable product, it would be of economic benefit to the farmer.

The objective of this study was to investigate the optimum yield and to characterize yellow pea protein extracted by acid and salt coagulations using Central Composite Rotatable Design.

Materials and Methods

Yellow peas (*Pisum sativum* L. Miranda) of sample grade US. No. 1 (based on USDA Standards) were grown in 1989 and provided by International Seeds Inc., Halsey, OR. Thirty-five kilograms of yellow peas were ground into medium particles and stored at 3^0C.

Methods. Protein Extraction. Proteins of yellow peas were extracted according to the temperature, pH level, and coagulation treatments. The protein extraction procedure is reported in Figure 3-1. Yields were calculated from total weight of freeze-dried products per 100 g yellow pea flour. Protein samples were powdered and kept in glass bottles and refrigerated (3^0C) until needed for protein characterizations.

Proximate Analysis. Yellow pea flour was analyzed for proximate composition by Columbia Lab. Inc. (Corbett, OR). Calcium was analyzed by ashing 2 g of proteinate at 525°C

for 24 h, dissolving in 3 mL of 3N HCl solution and diluting to 25 mL with redistilled water. The solution was analyzed using a Perkin Elmer 2380 atomic absorption spectrometer.

Nitrogen Determination. This N-determination was done using micro Kjeldahl method (AOAC, 1990), on all 13 treatments for the AP, MAP, and CAP. The results are presented as g protein/100 g sample, with a conversion factor nitrogen to protein of 5.7 (Columbia Lab. Inc., Corbett, OR.). Protein recovery was calculated based on total protein in the yield compared to that in 100 g yellow pea flour.

Amino acid composition. The composition was analyzed for AP, MAP, and CAP extractions at pH 9 and 25^0C, at the Center for Gene Lab (Oregon State University, Corvallis, OR). Samples were hydrolyzed in 6N HCl + 1.0% phenol before injecting onto a High Performance Liquid Chromatograph.

Electrophoresis. Electrophoresis of the proteinate used sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) according to the procedure of Laemmli (1970), using a protein concentration of 1.25 mg/mL, with 20 μL sample solution per well. Protein markers of known molecular weights (MW-SDS-70L, Sigma Chem. Co., St.Louis, MO) were used with bromophenol blue as the tracking dye.

SDS-PAGE for all samples was done with and without addition of 2-mercaptoethanol (2-ME) as a reducing agent, using 13% and 12% acrylamide gels, respectively.

Additionally, undenatured (without addition of SDS or 2-ME) samples were also electrophoresed to reveal the MW pattern of protein polymers. Electrophoresi was performed at a constant current of 40 ma or 35 ma per 2 gels for unreduced or reduced samples, respectively. Staining was done in 0.125% Coomassie brilliant blue R-250, 50% methanol, and 10% acetic acid, for 4 h. Destaining was done in 50% methanol, 10% acetic acid, for 2 h, then continued destaining in 5% methanol and 7% acetic acid, for 6 h. All assays were done at 25 ± 2 ^{0}C. Molecular weights of protein subunits were estimated from the plot of log MW versus the ratio of the distance traveled in comparison with the tracking dye. Gels were stored in a 7% glacial acetic acid solution until photographs were taken.

Experimental Design and Statistical Analysis. The experimental design used was a two-factor Central Composite Rotatable Design (CCRD) (Cochran and Cox, 1957), (Table 3-1), to predict maximum yields during protein extractions, and optimum characteristic measurements. Temperature ^{0}C (T) and pH (P) were independent (X) variables. The results obtained from extractions and protein characterizations were dependent (Y) variables. Statistical Analysis System (SAS Institute Inc., Cary, NC.) and Statgraph (Statistical Graphic Co., Rockville, Md.) programs were used to generate the ANOVA, parameter estimates, response surface analysis, canonical analysis, and ridge maximum of the responses.

Differences were considered statistically significant at P<0.10. The following model of quadratic polynomial regression was assumed for evaluating the individual Y-variables:

$$\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$$

Quadratic models were used to plot 3-dimensional response surfaces. Response surface analysis facilitated an understanding of the nature of responses obtained by graphically indicating maxima, minima, or saddles. All replications and measurements were done in duplicate.

Results and Discussion

Tables 3-2 and 3-3 present the proximate composition of yellow pea flour and calcium content of proteinates, and protein recovery during extraction, respectively. Table 3-4 presents parameter estimates for fitting the quadratic models for AP, MAP, and CAP on their extraction yields and nitrogen contents.

There are significant differences (P<0.01) in extraction yields in both AP and CAP, but not in MAP. Nitrogen content in all types of proteinate was not significantly different. Amino acid composition of three proteinates are in Table 3-5.

Protein extraction yields. The yield of acid-coagulated proteinate was significantly affected by changes in temperature and pH during extraction, as is represented in Figure 3-2A. The RSA has a hill shape with the maximum yield, 20.2%, at a treatment combination of $T=31.6^{0}C$ and $P=9.8$. The yield of CAP was affected ($P<0.001$) only by pH change. The RSA has a saddle shape (Figure 3-2C) with a maximum yield of 19.0% at a treatment combination of $T=27.0^{0}C$ and $P=10.8$. On the other hand, MAP was not significantly affected by either factor. Its RSA has a saddle shape (Figure 3-2B), with a tendency to high yield with treatment combinations of high T and high P. Its optimum treatment combination was $T=25.1^{0}C$ and $P=10.9$ which produced a yield of 16.8%. Significant positive relationships between pH and protein recovery were also reported by Shen et al. (1991) for soymilk coagulated with glucono delta-lactone or CaSO4.

Both salt-coagulated proteinates had 6-16% lower yields compared to acid-coagulated proteinates (Table 3-3). This might be because acid coagulation was done at pH 4.0, the lowest solubility of yellow pea protein, based on the preliminary work. Salt coagulations have a maximum yield at pH 6.5, since at this pH the carboxyl groups of aspartic and glutamic residues, and the imidazole groups of histidine residues are partially deprotonated (Kroll, 1984). Those groups bind with calcium or magnesium ions and cause the

protein to coagulate. Brooks and Morr (1984) reported that precipitation of soy protein extract at pH 4 caused precipitation of 11S and 7S proteins, while precipitation at pH 6.5 only precipitates the 11S fraction.

According to Lu et al. (1980), the salt coagulated protein is actually the protein isolate which is precipitated at a pH higher than its pI, as indicated by the pH of the whey, 6.5 for MAP and 5.5 for CAP, which explains the higher yield of acid-coagulated compared to salt-coagulated proteinates. Both acid and salt coagulations have higher yields than those reported by Gebre-Egziabher and Sumner (1983) or by Davis (1981), but lower than that reported by Naczk et al. (1986) or by Sumner et al. (1981). These might be due to differences in variety and processing conditions, which caused precipitation of phytic acid and carbohydrates such as pectins and gums, and phytic acid as has been reported (Shen et al., 1991; Thompson, 1987; Kantha et al., 1986; Brooks and Morr, 1984; McCurdy and Knipfel, 1990).

Nitrogen content. There were maximum values of 71.2, 72.4, and 69.5% protein (Table 3-3) for AP, MAP and CAP, respectively. None of the coagulation methods produced significant changes in nitrogen content with the changes of temperature and pH during extraction. This likely indicates that the protein being coagulated has similar purity and that the extraction and coagulation conditions had achieved

maximum levels for protein recovery.

As explained by Wang et al. (1983) and Aguilera and Garcia (1989), protein yield was significantly related to protein content in the extract solution. Nitrogen content of proteinate is about 3.7 times of that in yellow pea flour. Lu et al. (1980) also reported no difference in protein content for soy protein curds that were coagulated by acid, glucono lactone, or calcium salts.

Amino Acids. Amino acid compositions of the three proteinates are presented in Table 3-5. The amino acid composition was similar for all three proteinates with a hydrophilic:hydrophobic ratio of 60:40. All proteinates were low in cysteine and methionine, besides tryptophan as had been reported by other (Leterme et al., 1990). A similar distribution of amino acids also was reported by Okezie and Bello (1988) on winged bean protein isolate extracted at pH 10.0 and 12.0, or by Wang and Cavins (1989) on fractions obtained during tofu production.

Electrophoresis. SDS-PAGE photographs are reproduced in Figure 3-3. There were no apparent differences in electrophoresis patterns related to molecular weight (MW) subunits. All proteinates contained 7 major and 8 minor subunits with the highest MW=84 kD and the lowest MW=17 kD. Hsu et al. (1982) also reported 14 subunits ranging from 30 to 70 kD in dialyzed yellow pea protein isolate.

Electrophoretic patterns of undenatured samples (not

shown in figure) revealed the differences between protein polymers of AP and MAP or CAP samples. AP contained polymers that were not clearly separated on the gel, but had bands of different polymers. While MAP and CAP did not show any band at all, due to polymer size too large to enter the stacking gel. This could be the result of Mg- or Ca-bridging on the protein polymer.

Addition of 2-ME (Figure 3-3) gave a different MW pattern compared to the one without 2-ME (Figure 3-3). Electrophoretic patterns of samples without the reducing agent (Figure 3-3) showed that high MW subunits (>100 kD) which could not enter the stacking gel (4% acrylamide) which apparently dissociated into smaller subunits after 2-ME treatment, as it disappeared from wells on the stacking gel. The SDS-PAGE of unreduced samples resulted in the densest bands at subunits having MW=63 kD, which after treatment with reducing agent the densest bands were at subunits having MW=47 kD, and MW=24-26 kD.

Molecular weight patterns of undenatured proteinate samples indicated that forces, besides disulfide bonds, held the subunits together to form large protein polymers. These forces could be hydrophobic interactions, hydrogen bonds, electrostatic interactions, and/or salt bridges. The similarity in MW pattern of subunits with AP, MAP, and CAP samples, regardless of temperature and pH treatment combinations during extraction, indicated similarity in the

type of protein subunits that had been coagulated. The difference in resolution of protein polymers from undenatured samples indicated there might some bridging effects of magnesium or calcium ion that were absent from acid coagulation.

Data indicate that further exploration of yellow pea extracted proteinate is warranted. The coagulant used did not significantly influence yield or nitrogen content. Though there were no differences in the type of protein extracted, salt-coagulated proteinate contained calcium or magnesium, at 3.04% (w/w), which may be of added value, particularly if dietary calcium and magnesium are low in the general diet (Berner et al., 1990; Anonymous, 1991a). Amino acid composition of all three proteinates showed a similar pattern, with a ratio of 60:40 for hydrophilic to hydrophobic amino acids. The information obtained by this study will facilitate the development of vegetable-protein foods with high calcium content.

100 g YELLOW PEA FLOUR
  add 1000 mL H_2O
  blend for 10 sec
  liquify for 1 min

SLURRY I
  adjust with 2N HCl or NaOH to
  designated pH
  shake for 30 min at
  designated T °C
Centrifuge (2500 rpm, 10 min)

SUPERNATANT I   PRECIPITATE

adjust to (1)-pH 4.0 with 2N HCl add 500 mL
or (2)-pH 6.5 and add $MgSO_4$ liquify for
or (3)-add $CaCl_2$ 1 min

Centrifuge   SLURRY II

PRECIPITATE SUPERNATANT repeat as
       (discard)   for
            Slurry I

            Centrifuge

   SUPERNATANT II   PRECIPITATE
    repeat as for   (discard)
    Supernatant I

      Centrifuge

   PRECIPITATE   SUPERNATANT
           (discard)

COMBINE PRECIPITATE

  adjust to pH 8.0 with 2N NaOH

Freeze dry

1. ACID PROTEINATE (AP) or
2. Mg PROTEINATE (MAP) or
3. Ca PROTEINATE (CAP)
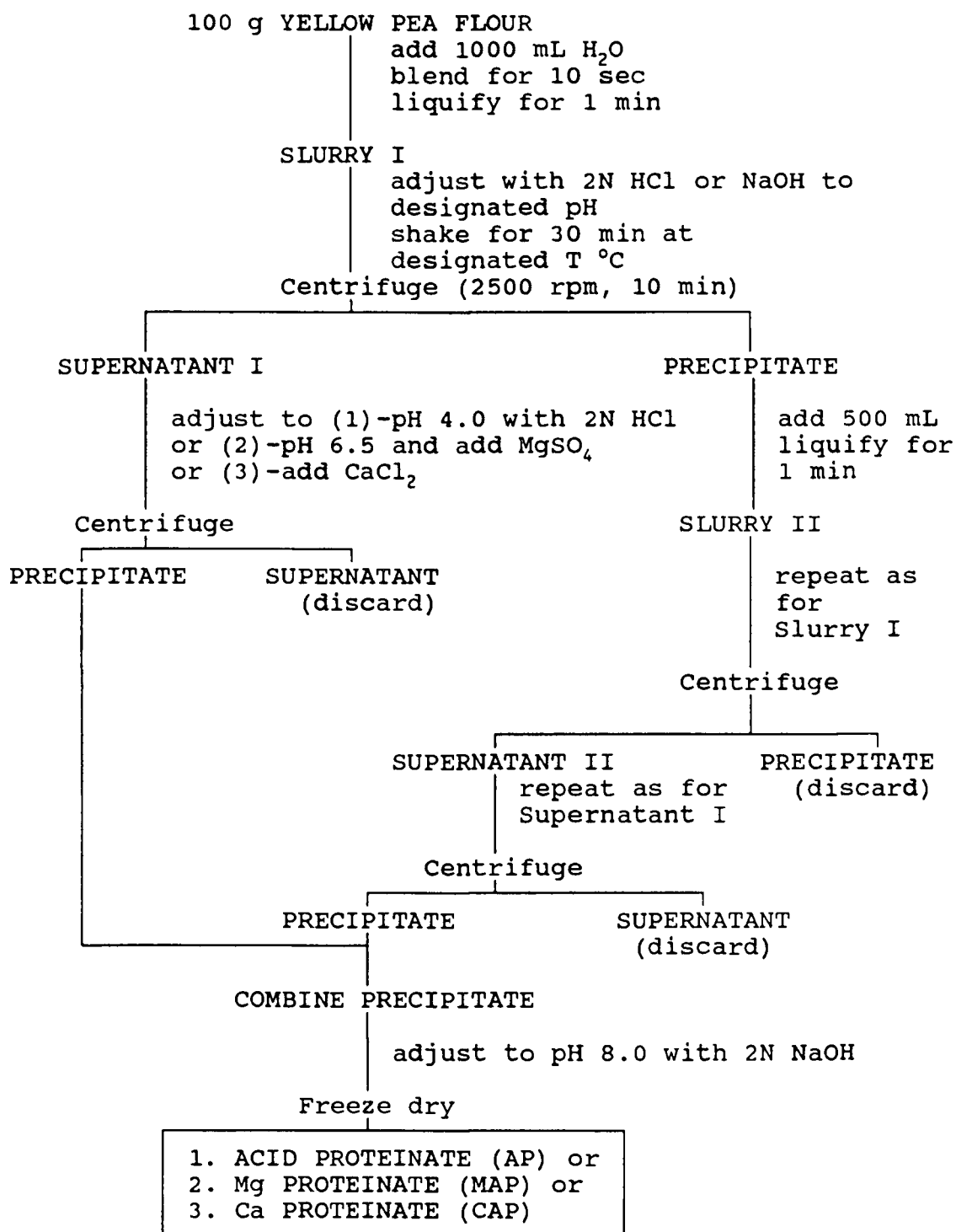
Figure 3-1. Extraction process of yellow pea protein using
  acid (pH 4.0), $MgSO_4$ anhydrous, or $CaCl_2$ $2H_2O$, as
  coagulant
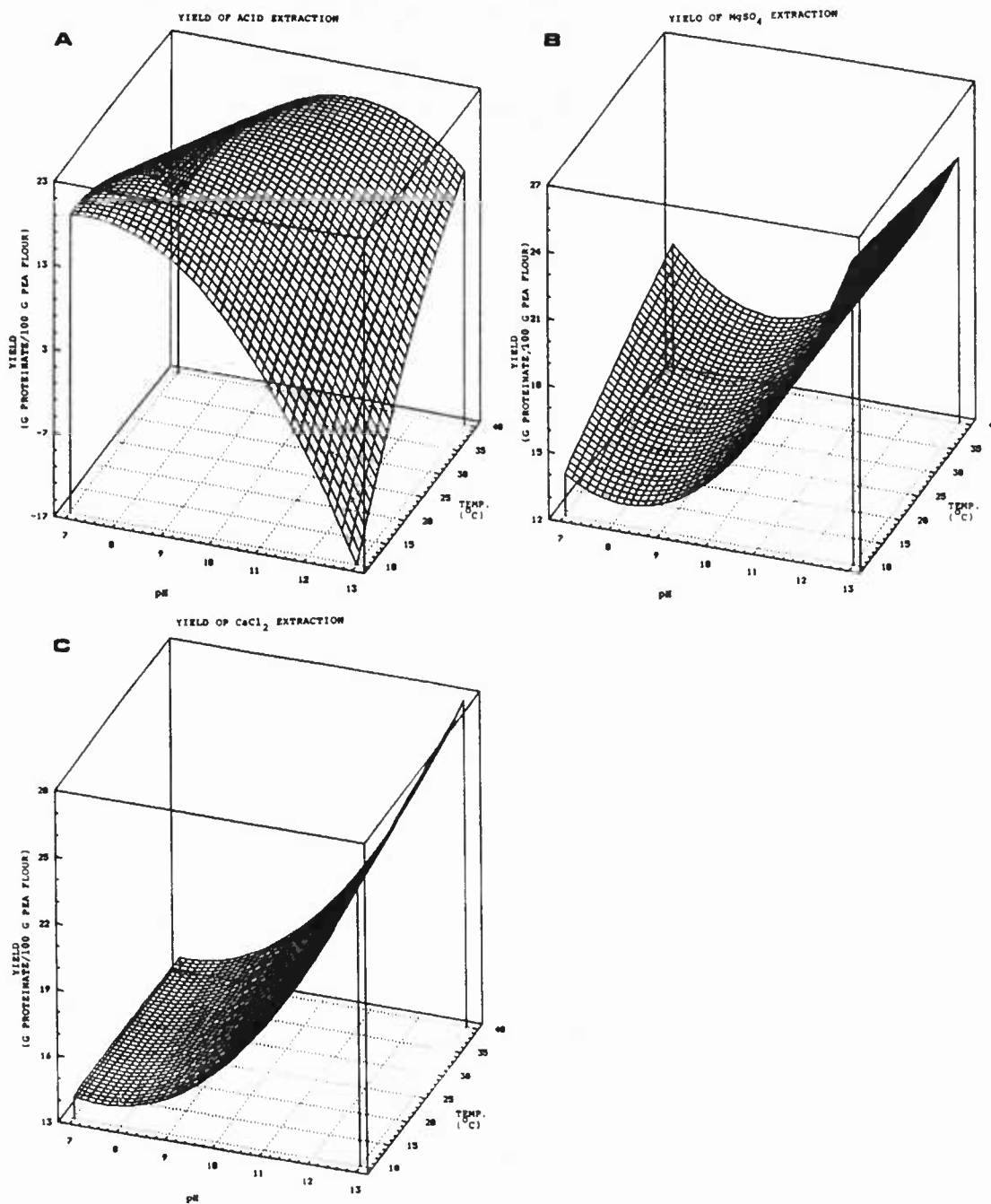
Figure 3-2. Three dimensional response surface models of
the yield from acid (A), magnesium (B), and
calcium (C) extractions
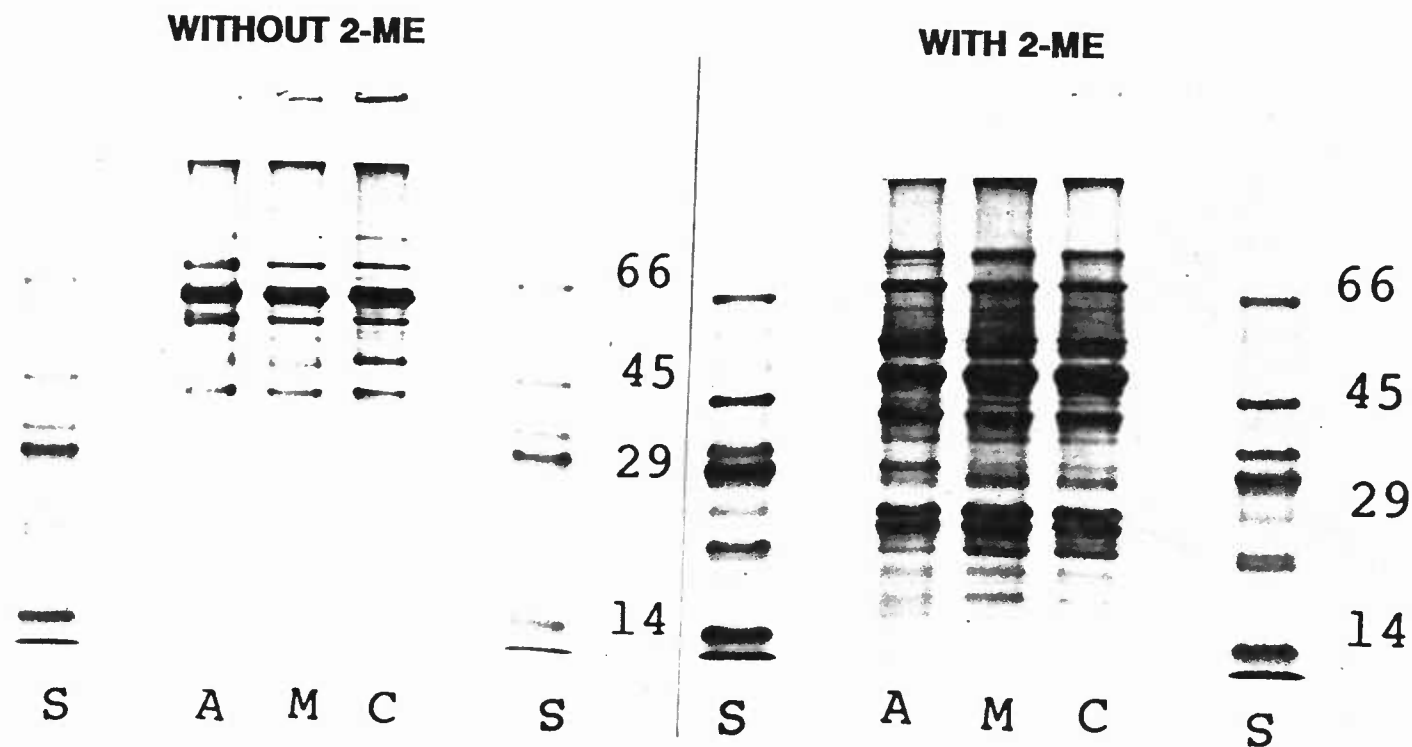
Figure 3-3. The electrophoresis patterns of acid (A), magnesium (M), and calcium (C) proteinates compared to molecular weight standard (S); without 2-mercapto ethanol (2-ME) addition, with 2-ME addition

Table 3-1. Visual display of the temperature-pH combinations used for the central composite rotatable design, with two independent variables[a,b]

DESIGN POINTS		-1.207	-1.000	0.000	1.000	1.207
	TEMPERATURE (°C)			pH		
		7.14	8.00	9.00	10.00	10.86
1.414	10			*		
1	15		*	*	*	
0	25	*		*** *		*
-1	35		*		*	
-1.414	40			*		

[a]Temperature-pH combination for each of coagulant types (Acid, 0.54% $CaCl_2$ $2H_2O$, 0.54% $MgSO_4$ anhydrous)
[b]Asterisks represent treatment of protein extraction for designated temperature °C (T) and pH (P)

Table 3-2. Proximate composition of yellow pea flour and
         calcium content of proteinates[a]

	per 100 g	
Energy	349.0 calories	
Protein (N X 5.7)	19.5 g	
Carbohydrate	64.2 g	
Fat	1.5 g	
Moisture	12.0 g	
Ash: Flour	2.8 g	
AP	6.3 g	(Ca= 0.03 g)
MAP	4.6 g	(Ca= 0.04 g)
CAP	8.7 g	(Ca= 3.04 g)

[a]AP is acid proteinate, MAP is magnesium proteinate, CAP is
calcium proteinate

Table 3-3. Protein content and recovery from extraction of
       yellow pea flour

Proteinate[a]	Yield (g/100 g)	Protein[b] (%)	Protein recovery[c] (%)
AP	20.2	71.2	73.8
MAP	16.8	72.4	62.4
CAP	19.0	69.5	67.7

[a]AP is acid proteinate, MAP is magnesium proteinate, CAP
calcium proteinate
[b]%N X 5.7
[c]Total protein in the yield compared to that in 100 g
yellow pea flour

Table 3-4. Quadratic regression model coefficients for yield and N content of three proteinate for substitution into equation[a], with T and P as independent variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Yield, %						
AP [c,d,e]	-29.954	-1.41	14.835	-0.016	-1.162	0.248
MAP	48.249	0.370	-9.099	-0.0003	0.569	-0.032
CAP[c,d]	38.572	-0.087	-6.372	-0.00002	0.410	0.012
N content, %						
AP	11.292	-0.21	0.896	0.001	-0.08	0.016
MAP[c]	7.122	0.214	0.818	0.0002	-0.018	-0.029
CAP[e]	-2.111	0.154	2.61	-0.0005	-0.114	-0.017

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$
AP is acid proteinate, MAP is magnesium proteinate, CAP is calcium proteinate
[b] Temperature is in ^0C
[c] CCRD is suitable for predicting the response
[d] pH (P) is significant variable
[e] Temperature (T) is significant variable

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Table 3-5. Amino acid compositions of three proteinates[a]
        (μg AA/ mg N)

	AP	MAP	CAP
Hydrophilic:	(64.2%)	(61.8%)	(62.1%)
ser	232.1	326.4	350.1
thr	172.8	242.2	266.4
tyr	122.3	179.0	180.0
asp+asn	547.0	742.4	798.6
glu+gln	858.1	1167.7	1252.0
lys	287.2	377.7	400.2
arg	440.2	645.2	686.6
his	113.7	158.6	175.3
Hydrophobic:	(35.8%)	(38.2%)	(37.9%)
cys	17.3	35.0	21.6
met	18.7	28.9	30.7
pro	102.4	323.0	322.6
gly	195.8	285.6	308.4
ala	212.9	308.2	336.2
val	261.6	371.1	399.3
leu	368.6	511.2	547.0
ile	215.5	294.2	317.5
phe	152.9	212.5	229.1
Total:	4319.1	6208.9	6621.6

[a]AP is acid proteinate, MAP is magnesium proteinate, CAP is calcium proteinate

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CHAPTER 4

FUNCTIONAL PROPERTIES OF ACID

AND SALT EXTRACTED PROTEINS OF YELLOW PEAS

(*Pisum sativum* L. Miranda)

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(*Pisum sativum* L. Miranda)

Abstract

Acid proteinate (AP), magnesium proteinate (MAP), and calcium proteinate (CAP), extracted from yellow pea flour at different pH (P) and temperature, ^{0}C (T) levels, were evaluated for functional properties. Nitrogen solubility index of AP is the highest (8.9 at pH 5.0 to 100% at pH 10.0-12.0), followed by MAP (3.4 at pH 5.0 to 97.3% at pH 12.0) and CAP (3.5 at pH 6.0 to 88.7% at pH 12.0). Maximum fat adsorption of AP, MAP, and CAP were 534% (T=30.3^{0}C, P=8.9), 522% (T=18.3^{0}C, P=10.0), and 510% (T=13.9^{0}C, P=10.3), respectively; with T being significant variable for AP and CAP, or T and P for MAP. Least gelation concentration for AP and MAP or CAP were 18% and 15%, respectively; with significant effect of P and T for AP. Emulsion capacity of AP, MAP and CAP were 62, 66, and 58 mL/g proteinate, respectively, with AP having the most stable emulsion.

Introduction

The composition of seed protein extracts such as nitrogen content, amino acid compositions and their conformation, will very much affect the functional properties of the protein (Elizalde et al, 1991; Kinsella, 1979; Okezie and Bello, 1988). These tests have also been used to assist in predicting the protein usefulness in food products, such as soy protein added to beef patties gave better yield and nutrient retention (Rice et al., 1989), or used in rice flour extrusion which gave better protein solubility of texturized products (Noguchi, et al., 1981). Extracted protein and solid in soymilk had been reported to affect yield and texture of tofu (Shen et al., 1991).

Since plant protein not only provides nutritional quality, but also provides desirable characteristics, there is a strong need to know and evaluate both qualities, so that it can be used effectively in the food product development. Some of the many other important criteria to develop viable texturized protein products are the protein should have optimum fat adsorption, emulsification properties, gelation, water absorption and binding (Kinsella, 1979).

Characteristics of protein extracted from yellow pea flour at different pH (P) and temperature (T) regimes have been reported (Soetrisno and Holmes, 1991). There were

similar nitrogen content, amino acid composition, and MW patterns among the three proteinates.

This study evaluated isolates extracted at 13 temperature-pH combinations for their nitrogen solubility, least gelation concentration (LGC), fat adsorption (FA), emulsion capacity (EC) and stability (ES). A small run was done to produce protein extrusion products from CAP as the strongest gel former.

Materials and Methods

Coagulated proteinate by acid (AP), magnesium (MAP), and calcium (CAP)) salts from yellow peas (*Pisum sativum* L. Miranda) flour was prepared (Soetrisno and Holmes, 1991), based on Central Composite Rotatable Design with temperature ^{0}C (T) and pH (P) as independent variables. Soybean oil was used for the measurement of fat adsorption (FA), emulsion capacity (EC) and emulsion stability (ES) of the proteinates.

Methods. <u>Nitrogen Solubility Index (NSI)</u>. The nitrogen solubility index of a 1% (w/v) proteinate solution in the range of pH 2.0-12.0 was determined based on the method of Thompson (1977), by measuring the nitrogen content (micro Kjeldahl, AOAC 1990) in the filtrate after centrifugation compared to that in the total solution.

<u>Least gelation concentration (LGC)</u>. Samples from 13 different extractions (Soetrisno and Holmes, 1991) were tested for LGC. The modified method of Tjahjadi et al. (1988) was used. Protein concentrations used were 20, 18, 16, and 14% for AP, and 15, 13, 11, and 9% for MAP or CAP. The LGC was determined as the lowest concentration of the proteinate that gave a gel that did not fall or run when the test tube was inverted.

<u>Fat adsorption (FA)</u>. The modified method of Tjahjadi et al. (1988), was applied using 0.5 g sample with 3 mL oil added.

<u>Emulsion capacity (EC)</u>. The method was modified from Okezie and Bello (1988). An aliquot of 0.5 g proteinate was dissolved in 12.5 mL 3% NaCL with 30 mL oil added while mixing. The emulsion was transferred into a 50 mL centrifuge tube, and held in a water bath (80^0C, 15 min), then centrifuged at 3000 rpm for 30 min. The volume of oil separated was used to calculate the EC.

<u>Emulsion stability (ES)</u>. The method of Okezie and Bello (1988) was used except that a 0.5 g sample was homogenized with 12.5 mL distilled water with addition of 25 mL oil. The emulsion was transferred into a 50 mL centrifuge tube, and total volume, total oil and liquid separated during standing ($25+2^oC$) were recorded at 0, 0.5, 2, and 6 h.

<u>Extrusion trial</u>. CAP sample (T=25^0C, pH=9.0) was selected. Six grams of proteinate were blended with 40 mL 1%

NaCl for 30 sec (VirTis homogenizer, speed 30), continued
with addition of 4 mL oil (1% addition) within 1 min. The
blended sample was held in a water bath (80°C) for 30 min.
Upon completion this cooked sample was transferred to a
potato ricer and pressed out and dried in a 95°C oven for 6
h. The product was informally evaluated by laboratory
personnel.

Statistical design and analysis. A two factor Central
Composite Rotatable Design (CCRD) (Cochran and Cox, 1957)
was employed to determine the interaction of P and T
combinations during protein extraction, on characteristic
measures, as described in Soetrisno and Holmes (1991).
Temperature ^{0}C(T) and pH (P) were independent (X) variables,
and the results obtained from NSI, LGC, FA, EC, and ES
measurements were dependent (Y) variables. Statistical
Analysis System (SAS Institute Inc., Cary, NC.) and
Statgraphics (Statistical Graphic Co., Rockville, Md.)
programs were used to generate ANOVA, parameter estimates,
response surface analysis (RSA), canonical analysis, and
ridge maximum and minimum of the responses. Differences were
considered statistically significant at P<0.10. All
measurements were done in duplicate. The following model of
quadratic polynomial regression was assumed for evaluating
the individual Y-variables:

$$\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$$

Results and Discussion

Nitrogen solubility index. The solubility of a product
is often dependent on the coagulant used to obtain it.
However, there was no significant effect of T-P combination
treatments during extraction on the NSI of AP or MAP (Table
4-1, 2), compared to that of CAP (Table 4-3). Figures 4-1A,
B, C, show the examples of their NSI profiles. NSIs of AP
and MAP proteinate were significantly affected by the
treatments during extraction. Acid coagulation caused higher
NSI of proteinate compared to Mg or Ca coagulation, which
were 8.9 at pH 5.0 to 100% at pH 12.0 compared to 3.4 at pH
5.0 to 97.3% at pH 12.0 or were 3.5 at pH 5.0 to 88.7% at pH
12.0, respectively. Similar result had been reported (Hsu et
al., 1982; Voutsinas et al., 1983) on NSI of yellow pea
protein isolate, with the lowest NSI at pH 4.0-6.0; but
higher than the NSI reported by Sumner et al. (1981) on pea
protein isolate from a similar preparation.

In the case of AP, NSIs at pH 7, 8, and 11 were high
when the treatment during extraction was done at
combinations of low T-low P, high T-high P, or high T-low P.
NSI at pH 2 was high at low T-low P or high T-high P
treatment combination only.

MAP had significantly higher NSIs at pH 2 and 3, when
extraction was done at low pH-all T combination treatments.
The minimum solubility at pH 6 was increased when the

extraction was done at high pH-all level T combination treatments. CAP had a wider pH range of minimum solubility than yellow pea flour, and it was not affected by either factor during extraction.

However, the practical significance of this may be limited. For example, the 100% solubility for acid-coagulated proteinate was at a pH 10 to 12. Rarely are foods at this pH. In the range of pH 5-7, the acid (Figure 4-1A), magnesium (Figure 4-1B), and calcium (Figure 4-1C) proteinates had a NSI of 8.9-81.3%, 3.4-17.2%, and 3.5-9.2%, respectively. The lower NSI for MAP and CAP were probably due to salting-out-denaturation during protein coagulation.

Least gelation concentration. There were very distinct differences between LGC of AP, MAP, and CAP. Table 4-4 data showed the regression coefficients were zero for CAP. The MAP and CAP could form a strong gel at all concentrations tested, only the amount of water held within the matrix gel made the difference. Almost all MAP and CAP from all extraction treatments had 15% LGC, in which all water was held within the gel system, except MAP extracted at $T=15^0C$, $P=8.0$ which had 13% LGC. Both RSA of MAP and CAP showed flat surfaces (not shown in figure), due to no effect on LGC value of P or T during extraction, probably due to the same concentration of divalent-ions in all proteinates which played the chief role in water imbibing activity.

LGC of acid-proteinate was significantly ($P<0.001$)

affected by the P and T levels during protein extraction. It was 18.0% (T=22.7^{0}C, P=9.6) (Table 4-4, Figure 4-2) as the least concentration to form a gel. Low P-high T, and high P-low T combinations gave higher LGC values.

Fat adsorption (FA). Acid proteinate gave maximum FA=534% (T=30.3^{0}C, P=8.9), which is more than twice the value previously reported (Sumner et al., 1981). There was a significant (P<0.01) effect of T level during extraction on the FA (Table 4-4). The lower T had significantly lowered adsorption ability. The FAs were 522% (T=18.3^{0}C, P=10.0) and 510% (T=13.9^{0}C, P=10.3), for MAP and CAP, respectively, with significant (P<0.10) effects of T and P for MAP and of T only for CAP (Table 4-4). Extreme combinations, low P-low T and high P-high T, caused decreases FA for MAP, but only the high P-high T combination caused decreases FA for CAP. These high FA values were probably due to denaturation of protein during extraction and/or salt coagulation, which brings about the exposure of lipophilic or hydrophobic residues; resulting in increased lipid-protein complexes (Kinsella, 1979).

Emulsion capacity (EC). There were significant (P<0.10) increases in EC with decreasing T or increasing P during extraction of AP (Table 4-5). The maximum EC value was 62 mL/g proteinate at T=17.7^{0}C, P=10.6. Only decreasing P significantly (P<0.10) increased the EC of MAP, with maximum value of 66 mL/g (T=24.6^{0}C, P=7.9). The EC of CAP was not

affected by either factor during extraction; its maximum value was 58 mL (T=27.4^{0}C, P=8.5). All EC values were higher than the value reported by Sumner et al. (1981). This is mostly due to the ability of proteinates to bind fat, in addition to the higher concentration of protein in the dispersion (Mine et al., 1991). The pH of dispersion was 7.5-8.0; as opposed to the acidic pH that had been reported to give highest emulsion activity.

Emulsion stability (ES). ES as measured by volume changes during standing have maximum of 79, 78, and 76 mL/g proteinate for AP, MAP, and CAP emulsion, respectively. Slight decrease in volume was observed after 6 h for MAP, and was no change for the other two (Table 4-5). Initial volume before homogenization was 75 mL. Emulsion of AP was very stable for more than 6 h (Table 4-6) with very thick mayonnaise-like texture, while the emulsions of MAP and CAP were stable up to 0.5 h only as measured by retained water, with thick dressing-like viscosity. These results were expected as AP formed a more viscous solution than MAP and CAP during protein dispersion, which indicated that AP has higher ability to imbibe water.

Retained water in MAP or CAP emulsion after 2 h standing (Table 4-6) was affected by both increasing T (P<0.10) and decreasing P (P<0.001), with the same maximum volume of 26 mL/g proteinate, at T=36.1^{0}C, P=7.8 for MAP and at T=37.4^{0}C, P=8.0 for CAP. When the stability was measured

by retained oil, AP emulsion was stable up to 6 h, while MAP and CAP emulsion were stable up to 2 h (Table 4-6). Retained oil in MAP and CAP emulsions were not significantly affected by either factor during extraction. Maximum volumes of retained oil in AP, MAP, and CAP emulsion were 50, 49, and 55 mL, respectively, after 6 h standing. Figure 4-3A and B show the differences on response surface model for emulsion stability of MAP when measured by retained oil and retained water after 6 h standing.

Extrusion trial. CAP, as the strongest gel former, was the only product used for extrusion. The product, which had 10% added fat gave a good product, based on texture, flavor, and overall acceptance. The only slight objection was that the product had a slightly dark color, which may have been due to the oven drying method after extrusion.

Descriptive evaluation. Although objective color measurements were not taken, there were consistent differences in the proteinates. The dry acid-coagulated proteinate was shiny-white with fluffy particles. Mg-coagulated proteinate was white color with denser particles, while Ca-coagulated proteinate was creamy-white with the densest particles.

Summary. The functional properties of proteinates may be used to predict the application of these protein in food systems. Laboratory scale extractions of yellow pea flour can already recover more than 62% of its protein. Although

acid or salt coagulation gave similar nitrogen content, their solubilities, gelation properties, and emulsion stabilities were different.

Acid proteinate was easily dispersed and had high solubility in low pH, make it possible to be incorporated in beverages or soups; together with salt proteinates can also be used as animal protein replacer or extender. Based on these data of functional properties, all the three proteinates have marketability, especially if the final exploration on food product development is accomplished.
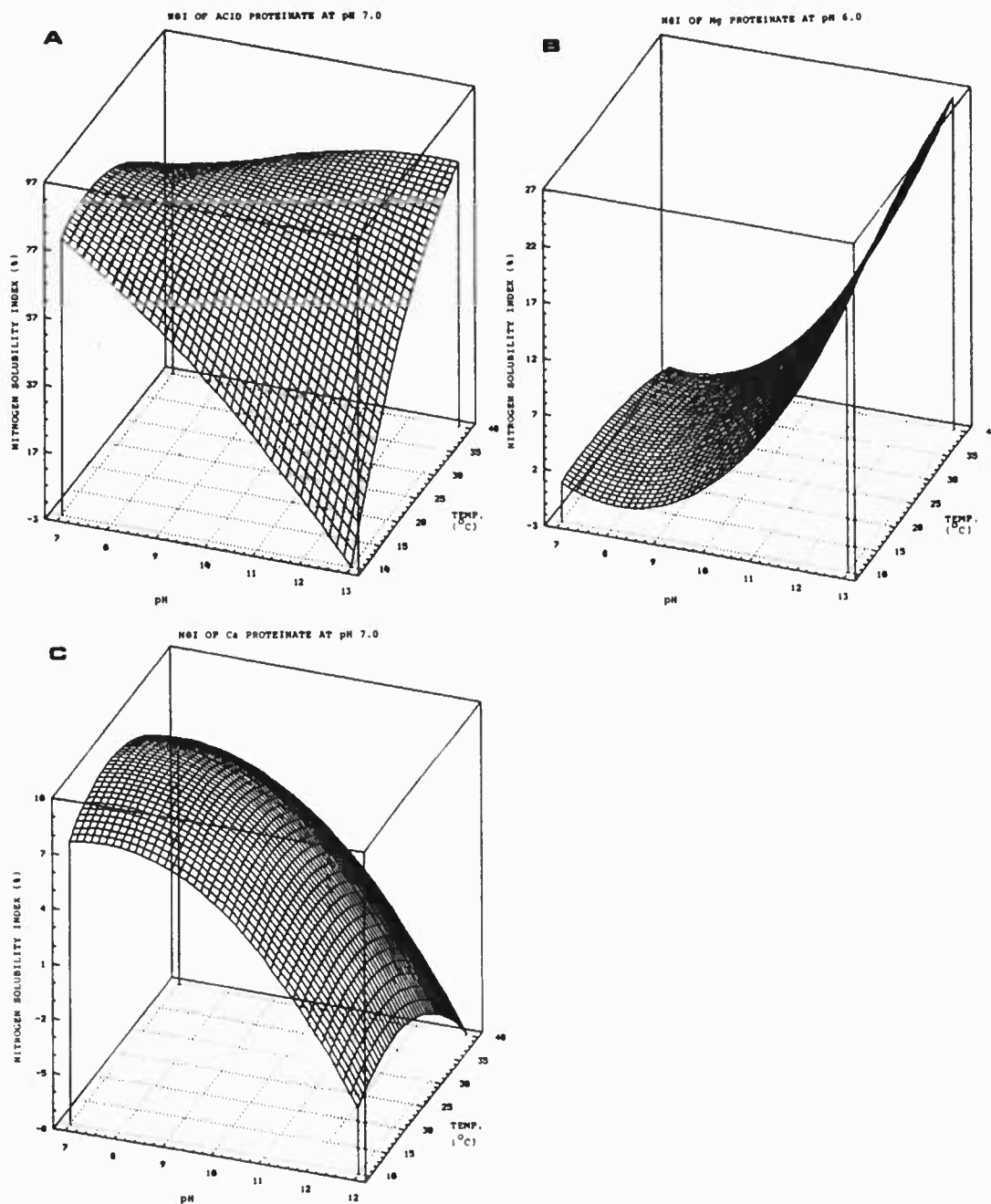
Figure 4-1. Three dimensional response surface models of
the nitrogen solubility index of acid (A), magnesium
(B), and calcium (C) proteinates at pH 6.0 or 7.0
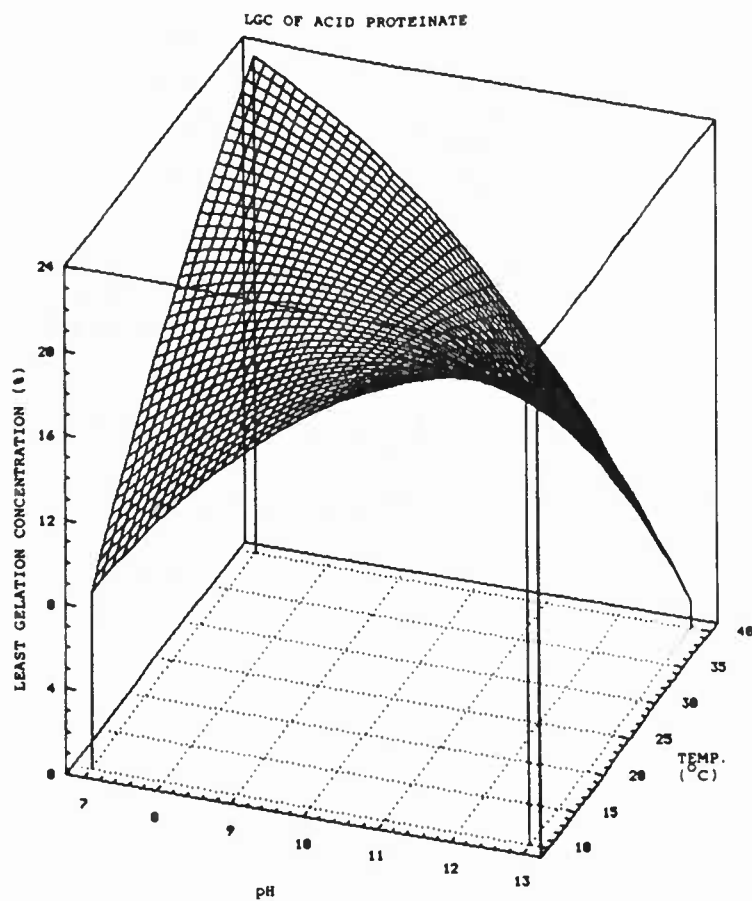
Figure 4-2. Three dimensional response surface model of
least gelation concentration of acid proteinate
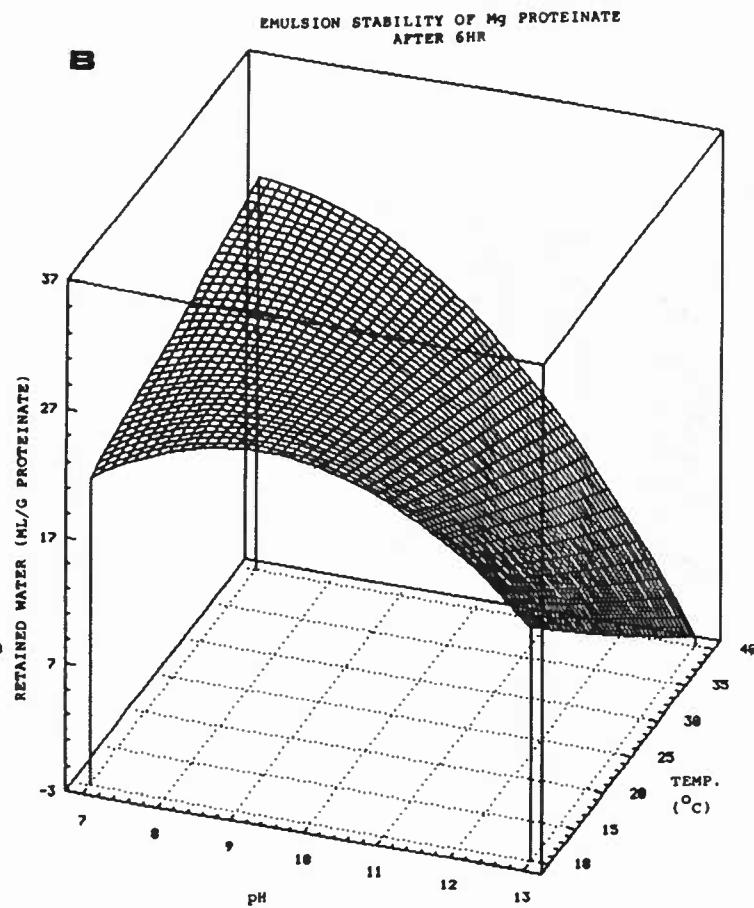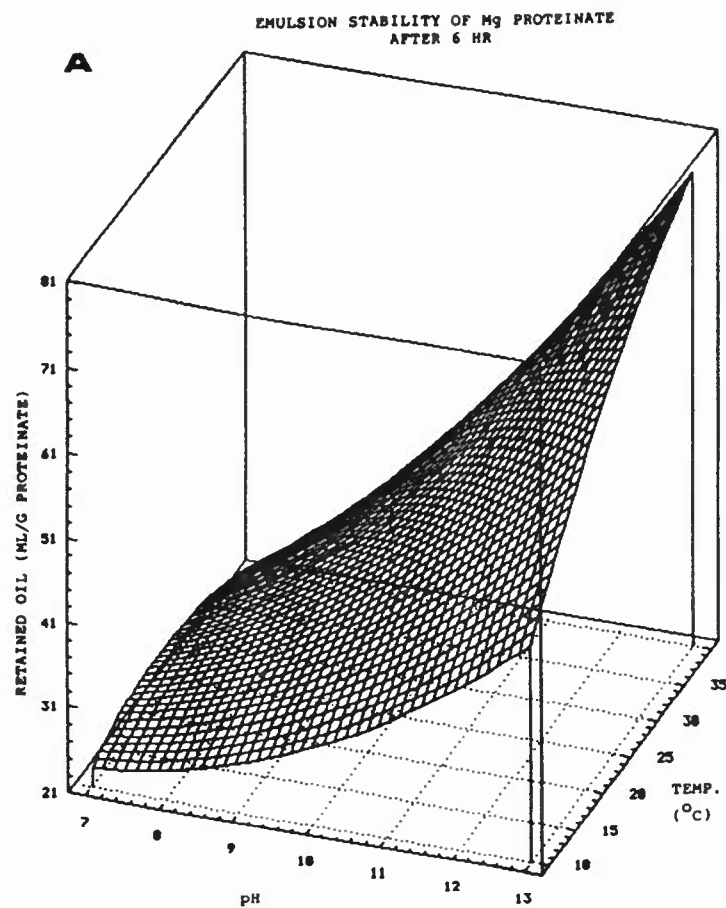
Figure 4-3. Three dimensional response surface models of emulsion stability of magnesium proteinate as measured by retained oil (A), and retained water (B) after 6 h

Table 4-1. Quadratic regression model coefficients for
        NSI of acid proteinate for substitution into
        equation[a], with temperature and pH as independent
        variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Nitrogen Solubility Index (NSI), %						
at pH 2[c,d]	356.278	-2.287	-49.972	-0.069	1.517	0.650
pH 3[c]	189.178	-4.214	-12.345	-0.054	-0.487	0.778
pH 4[c]	-48.933	-6.619	24.985	-0.032	-2.238	1.010
pH 5[c]	123.342	-0.926	-25.983	-0.007	1.346	0.132
pH 6[c]	-54.404	-3.186	43.677	-0.095	-3.846	0.728
pH 7[c,e]	141.465	-1.999	-6.372	-0.058	-0.655	0.578
pH 8[c,d,e]	197.127	-0.373	-20.018	-0.067	0.177	0.440
pH 9[d,e]	189.781	-2.383	-12.476	-0.063	-0.483	0.642
pH 10[c]	323.313	-0.836	-48.202	-0.095	1.716	0.628
pH 11[c,e]	205.918	0.603	-25.284	-0.070	0.801	0.362
pH 12[c]	178.058	-3.224	-8.768	-0.012	-0.169	0.445

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$
AP is acid proteinate, MAP is magnesium proteinate, CAP is
calcium proteinate
[b] Temperature is in ^0C
[c] CCRD is suitable for predicting the response
[d] pH (P) is significant variable
[e] Temperature (T) is significant variable

Table 4-2. Quadratic regression model coefficients for NSI of magnesium proteinate for substitution into equation[a], with temperature and pH as independent variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Nitrogen Solubility Index (NSI), %						
at pH 2[c,d]	-116.047	2.078	42.600	-0.042	-2.747	0.025
pH 3[c,d]	-52.353	5.288	19.767	-0.042	-1.048	0.395
pH 4[c,d]	-508.290	19.540	73.338	-0.064	-2.019	-1.790
pH 5[c]	-8.123	-0.878	5.339	-0.007	-0.504	0.130
pH 6[c,d]	63.357	-0.147	-15.645	-0.005	0.929	0.045
pH 7[e]	190.451	-1.428	-35.233	-0.021	0.245	1.588
pH 8[c]	-107.630	-4.437	45.621	0.003	-3.169	0.392
pH 9[c]	-174.171	0.169	58.901	-0.004	-3.756	0.028
pH 10[d]	-146.605	-3.572	59.535	-0.008	-4.096	0.420
pH 11	592.034	-6.205	-96.684	-0.013	4.175	0.775
pH 12[c]	212.546	-1.651	-23.981	0.002	1.051	0.210

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{12}*P*P + B12*T*P$
[b] Temperature is in ^0C
[c] CCRD is suitable for predicting the response
[d] pH (P) is significant variable
[e] Temperature (T) is significant variable

Table 4-3. Quadratic regression model coefficients for NSI of calcium proteinate for substitution into equation[a], with temperature (T) and pH (P) as independent variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Nitrogen Solubility Index (NSI), %						
at pH 2	116.581	1.432	-12.955	-0.046	0.377	0.072
pH 3[c]	-201.170	3.207	52.356	-0.066	-3.144	-0.042
pH 4[c]	-344.563	4.467	68.128	-0.061	-3.421	-0.192
pH 5	196.389	-2.735	-36.538	0.002	1.694	0.278
pH 6[c]	-66.530	0.711	12.691	0.003	-0.562	-0.090
pH 7[c]	-22.069	0.548	6.647	-0.012	-0.428	-0.005
pH 8[c]	60.923	0.556	-11.768	-0.006	0.593	-0.020
pH 9	213.318	1.545	-44.607	-0.027	2.326	-0.038
pH 10	315.657	2.935	-71.742	-0.005	4.263	-0.280
pH 11[c]	111.515	1.368	-10.244	-0.065	-0.131	0.235
pH 12[c]	416.553	-4.770	-65.072	-0.030	2.669	0.720

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}T*T + B_{22}*P*P + B_{12}*T*P$
[b] Temperature is in ^0C
[c] CCRD is suitable for predicting the response

Table 4-4. Quadratic regression model coefficients for LGC
and FA of AP, MAP, CAP for substitution into equation[a]
with temperature and pH as independent variables[b].

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Least gelation concentration (LGC), %						
AP [c,f]	-62.900	2.110	11.880	-0.004	-0.380	-0.200
MAP	-1.770	0.520	2.020	-0.001	-0.030	-0.050
CAP[c]	15.000	0.000	0.000	0.000	0.000	0.000
Fat Adsorption (FA), %						
AP [d,f]	-1950.800	59.850	356.150	-0.510	-14.480	-3.280
MAP[d,c,f]	-1521.460	78.120	198.320	-0.420	-1.290	-6.210
CAP[d,f]	105.300	38.610	-5.570	-0.200	4.730	-3.730

[a]$\hat{Y} = A + B_1*T + B_2*P + B_{11}T*T + B_{22}*P*P + B_{12}*T*P$
AP is acid proteinate, MAP is magnesium proteinate, CAP is calcium proteinate
[b]Temperature is in ^0C
[c]Stationary point of the RSA is in flatness
[d]CCRD is suitable for predicting the response.
[e]pH (P) is significant variable
[f]Temperature (T) is significant variable

Table 4-5. Quadratic regression model coefficients for
emulsion capacity and total volume of emulsion for
substitution into equation[a], with temperature and pH as
independent variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Emulsion Capacity (EC), mL oil/g proteinate						
AP [d,e]	-20.938	2.128	10.507	-0.005	-0.195	0.252
MAP[d]	-547.476	6.070	136.068	-0.084	-0.245	8.212
CAP	-820.565	9.474	176.069	-0.189	0.100	-10.510
Total volume, mL, at 0 h:						
AP [c]	1.306	1.664	12.854	-0.006	-0.150	-0.533
MAP[c]	96.216	0.222	-4.623	-0.005	0.000	0.238
CAP[c]	97.738	-0.339	-4.241	-0.002	0.050	0.176

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$
AP is acid proteinate, MAP is magnesium proteinate, CAP is calcium proteinate
[b] Temperature is in ^0C
[c] CCRD is suitable for predicting the response
[d] pH (P) is significant variable
[e] Temperature (T) is significant variable

Table 4-6. Quadratic regression model coefficients for
emulsion stability for subtitution into equation[a], with
temperature and pH as independent variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
Retained Oil, mL/g proteinate:						
After 0 and 0.5 h:						
AP[c], MAP[c], CAP[c]	50.000	0.000	0.000	0.000	0.000	0.000
After 2 h:						
AP [c]	50.000	0.000	0.000	0.000	0.000	0.000
MAP[d]	200.334	-3.171	-24.461	0.011	0.310	0.849
CAP	73.808	-1.048	-1.734	-0.005	0.150	-0.163
After 6 h:						
AP	48.770	0.047	0.165	0.000	-0.005	-0.003
MAP[d]	51.216	-0.485	-8.556	-0.016	0.175	0.534
CAP	239.256	-4.949	-28.360	0.002	0.575	0.670
Retained Water, mL/g proteinate:						
After 0 h:						
AP[c], MAP[c], CAP[c]	25.000	0.000	0.000	0.000	0.000	0.000
After 0.5 h:						
AP[c]	25.000	0.000	0.000	0.000	0.000	0.000
MAP	16.185	0.045	2.053	-0.001	0.000	-0.124
CAP[c]	13.316	0.081	2.592	0.000	-0.010	-0.142
After 2 h:						
AP[c]	25.000	0.000	0.000	0.000	0.000	0.000
MAP[c]	-24.194	0.419	10.700	0.000	-0.050	0.573
CAP[c]	-21.735	0.325	10.369	0.000	-0.040	-0.567
After 6 h:						
AP[c]	25.000	0.000	0.000	0.000	0.000	0.000
MAP[d,e,f]	-53.666	1.107	15.901	0.001	-0.135	-0.776
CAP[e,f]	-127.473	1.160	33.163	-0.002	-0.125	-1.801

[a] $\hat{Y} = A + B_1*T + B_2*P + B_{11}*T*T + B_{22}*P*P + B_{12}*T*P$
AP is acid proteinate, MAP is magnesium proteinate, CAP is
calcium proteinate
[b] Temperature is in ^{0}C
[c] Stationary point of the RSA is in flatness
[d] CCRD is suitable for predicting the response.
[e] pH (P) is significant variable
[f] Temperature (T) is significant variable

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CHAPTER 5

IMPLICATIONS FOR THE FUTURE

The development of a extracted yellow pea protein
product of 68-70% protein and less than 1.5% which has the
properties required for a marketable textured vegetable
protein product is possible. Based upon this knowledge, a
variety of applications for this protein extraction may be
developed. One of these may be an extruded vegetable protein
product. Optimization research is still necessary using
various extrusion conditions, such as different
temperatures, times, pressures, and die sizes.
Additionally, the interaction of other ingredients need
investigation. Ingredients such as polysaccharides (sugar,
starch, gums), flavorants, and colorants may increase the
acceptability of the flavor, appearance and texture.
Incorporation of other proteins, to complement the
sulfur-containing amino acids and tryptophan, may enhance
the nutritive value.

High solubility in lower pH, high ability to adsorb
free fat droplets, emulsions with high stability, and the
ability to form a heat-induced gel make this protein extract
usable in various food systems. Acid proteinate can be
incorporated into beverages, mayonnaise, salad dressing; or
as salt coagulated proteinates, they can be used as meat or

fish extenders in sausages, made into cheese analogs, spreads, soups, cakes, bread, or doughnuts.

The presence of factors with physiological activity and nutritional adequacy needs to be investigated. Based on other vegetable protein concentrates, the presence of impurities such as antitrypsin, oligosaccharides, phytic acids, or lipoxydases should be evaluated. Not only would the presence of oligosaccharides, raffinose, stachyose, and verbacose cause digestion problems but it would decrease acceptability. If these are present, large scale equipment for aqueous extraction followed by membrane filtration for removal may be used. The added cost of processing would increase product cost; however, with current extractions, the proteinate is considered a protein concentrate (protein>60%), and already has good functional properties. Protein digestibility and completeness are also nutritional criteria to be considered. The protein efficiency ratio (PER) of the proteinate should be considered and evaluated.

This research project was based on a yellow pea (*Pisum sativum* L. Miranda) variety of approximately 21% protein. It successfully produced a proteinate that has marketability. There are other yellow pea varieties with even higher protein content. The potential market for yellow pea vegetable protein sources is tremendous if the final phase of food product development is accomplished.

CHAPTER 6

SUMMARY OF RESULTS

Coagulation of yellow pea protein produces a viable proteinate with a yield of 16 to 20%, which recovered 62 to 74% of protein in the flour. Yield (g/100 g flour) of AP was affected by different P-T combinations, while MAP or CAP was not. This is understandable since salt coagulation was done at pH 6.5, which only precipitated the 11S fraction (Brooks and Morr, 1984).

Amino acid composition showed similar ratios of hydrophilic to hydrophobic (60:40) for all proteinates, with AP containing the lowest amount; which might be due to losses during hydrolysis. The molecular weight patterns were no different among the three proteinates, with MWs from 17-84 kD per subunit; S-S bonds were mostly at subunits with MW 63->100 KD. CAP contained 3.04% Ca, compared to only 0.03% for both AP and MAP.

All proteinates contained similar amounts of N (12.3-12.7%), with nitrogen to protein conversion factor 5.7. Although N was similar, the proteinates had different solubilities as the result of different coagulants. AP had the highest solubility (9-81%), while the other two were 4-17% over the range of pH 5-7.

Least gelation concentration was 18% for AP and 15% for

MAP or CAP. AP had maximum fat adsorption of 534%, values for MAP and CAP were 522 and 510%, respectively. All proteinates had similar emulsion capacities (58-66 mL oil/g protein) with AP as the most stable.

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APPENDICES

Appendix 1. Extraction methods reported in selected
references

Nichols and Cheryan, 1981

-**Soy Isolate**: extract defatted flour(1:9) in H_2O, at pH

9.0 w/ 1N NaOH, fed to centrifugal desludger.

Resuspend residues w/ H_2O (1:10), done 3 times.

Collect supernatant adjust to pH 7.0 w/ 6N HCl, put

into ultrafilter w/ hollow unit fiber.

Wang, 1981

-extract **soy flake** (1:10) in H_2O, sonicated in chilled

for 8 min, centrifuge at 10000g/15 min. Take

supernatant, lyophilized.

Kitabatake and Doi, 1982

-extract **defatted soy flour** (1:10) in H_2O, centrifuge

at 12000g/10 min. Bring filtrate to pH 4.5,

recentrifuge at 100001g/10 min. Adjust precipitate to

pH 7.0 and freeze dried.

Saio et al.,1982

-extract 3 g **soy flour** in 20 mL H_2O, refrigerate

overnight, add 10 mL 0.1M phosphate buffer (pH 7.6),

1.2M NaCl, 3mM DTT. Stir in RT/60 min, centrifuge at

10000g/30 min. Add acetone to filtrate 1.5 fold at -

20^0C dropwise, centrifuge at 10000g/30 min in cold, 2

times. Dried precipitate in vacuum oven.

Wang et al., 1983

-make **soybean** slurry (1:10) w/ H_2O, boil for 15 min,

filtered w/ 4-layer cheese cloth. Cooled to 70^0C, add

400 mL 10% $CaSO_4$ $2H_2O$. Set for 10 min, press in cheese

cloth.

Brooks and Morr, 1984

-**7S fraction**: extract **defatted soy flour** (1:20) in

dilute Tris, centrifuge and adjust filtrate to pH 7.8.

Freeze dried.

Honig et al., 1984

-extract **defatted soybean flakes** (1:5 and 1:10) in H_2O

and in alkali, at pH 8.0 w/ 2N NaOH for 40-60 min.

Centrifuge at 10000 rpm (16000rcf)/20 min. Add filtrate

w/ 2N HCl to ph 4.5, centrifuge. Wash ppt. then freeze

dried as Isoelectric protein isolate. Or: adjust ppt.

to pH 8.0, freeze dried as Neutralized PI.

-**11S fraction**: add 0.03M Tris-HCl to **defatted soy**

flour, at pH 8.0. Centrifuge at RT, bring filtrate to

pH 6.4, recentrifuge at $2-5^0C$, collect ppt.

-**2S fraction**: bring the filtrate to pH 7.6 w/ 0.03M

Tris-HCl, extract is 2S.

-**7S fraction**: add ppt. w/ Tris-HCl to pH 6.2,

centrifuge. Put filtrate to Sepharose 6B to purify 7S.

Kroll, 1984

-extract **defatted soy flour** (1:10) in

dilute NaOH, pH 12 at RT/1 h, centrifuge at 2000 rpm or

600g/20 min. Dialyzed against H_2O for 100 h at 4^0C,

change H_2O after 60 h Add ppt. w/ dilute HCl to pH 4.5,

centrifuge at 2000 rpm/20 min then freeze dried the

ppt.

Sato et al., 1986

-extract **defatted soy flour** (1:20) in H_2O at RT,

centrifuge at 15000g/25 min at 5^0C, collect the **water**

soluble protein. Add (1:20) 0.5M NaCl to residue,

centrifuge at RT, done 4 times, as **NaCl soluble**

protein.

Elizalde et al., 1988

-make slurry of **defatted soy flour** in H_2O, pH 8.0,

centrifuge. Bring filtrate to pH 4.5 w/ HCl, wash

precipitate w/ H_2O, adjust to pH 7.0 w/ NaOH, spray

dried.

Thompson and Erdman, 1988

-**Protein concentrate**: extract **defatted soy flour** (1:10)

in H_2O at 40^0C/30 min. Add 3N HCl to pH 4.5 and store

overnight at 7 C. Remove whey, repeat 3 times, freeze

dried. Or: add 20% NaOH to pH 7.0, freeze dried

-**Water wash concentrate**: extract defatted flour

(55g:65mL) in H_2O, spread paste in st. steel pan,

autoclave at 121^0C/5 min. Grind and disperse in H_2O

(1:10), heat suspension to 82^0C/15 min. Remove whey,

repeat 2 times. Centrifuge and Freeze dried.

-**Tofu Analog**: extract defatted flour (1:10) in H_2O,

heat to $90^0C/5$ min, cooled to 70^0C. Add $CaSO_4$ (2.25 g +

18 mL H_2O for 420 mL slurry), mix rapidly. Incubate at

$70^0C/10$ min. Broke curd, centrifuge at 100g/5 min.

Resuspend ppt., repeat 2 times. Freeze dried ppt.

-**Protein Isolate**: extract whole flour (1:10) in H_2O,

bring to pH 9.0 w/ 20% NaOH, stir at $35^0C/45$ min.

Passed through centrifugal desludger. Adjust extract to

pH 4.5 w/ 3N HCl, stored overnight at 7^0C. Remove whey,

done 3 times, freeze dried the ppt., or add 20% NaOH to

pH 7.0, freeze dried.

Thompson and Fosmire, 1988

-extract **soy flour** (1:10) in 60% ethanol, heat to

$57^0C/30$ min. Remove supernatant, replace w/ 60%

ethanol, repeat 2 times. Centrifuge the protein rich

sludge, dried the ppt. to remove residual ethanol.

Yao et al., 1988

-extract **defatted soy flour** (1:14) in H_2O, adjust to pH

8.5 with 6N NaOH, 30 min; centrifuge at 2000 rpm/15

min. Adjust filtrate to pH 4.5 with 6N HCl, 30 min,

centrifuge. Suspended precipitate in H_2O, pH 7.0,

freeze dried. Or: to collect 11S, add 0.03M Tris buffer

to ppt. (pH 6.4) at 1^0C, centrifuge. Bring filtrate to

pH 4.8, 7S will be ppted.

Sumner et al., 1981

-extract **yellow pea flour** (1:5) in H_2O, add 1N NaOH to

pH 9.0, stir 20 min, centrifuge 1000g/20 min. Done 2

times. Bring combined filtrate to pH 4.5, centrifuge
and redisperse ppt. w/ 100 mL H_2O at pH 9.0,
reprecipitate, and dried. Or: add 1N NaOH to ph
6.5-7.0, freeze dried.

Hsu et al., 1982

-extract flour **(yellow pea, lentils, faba bean)** (1:10)
in H_2O, to pH 8.5 w/ 1N NaOH at 40^0C/30 min, done 2
times. Centrifuge at 220g at 10^0C/10 min. Combined
filtrate, add 1N HCl to pH 4.5, centrifuge at 1570g at
10^0C/10 min. Wash ppt. w/ H_2O, add 1N NaOH to disperse
ppt. to pH 7.5. Dialyzed in running tap water at
10^0C/24 h, lyophilized.

Gebhre-Egziabher and Sumner, 1983

-extract **field pea flour** (100 g/500 mL) in H_2O, add
0.2% CaO to pH 8.8-9.0, centrifuge at 100g/20 min.
Heat to $95-100^0C$/20 min, cooled to $75-80^0C$ for 2 sec.
Coagulate w/ 0.54% $CaCl_2$, HOAc, $CaSO_4$.

Ulloa et al., 1988

-extract **chickpea flour** (1:8 to 1:20) in H_2O, at pH 2-
12. Apply continuous centrifugation. Soluble solids
were concentrated in hollow fiber ultrafiltration unit.
Spray dried.

Thompson, 1977

-**Protein Isolate**: extract **mungbean flour** (1:15) in H_2O,
add 1N HCl or NaOH to pH 1-12. Shake in waterbath
25^0C/20 min, centrifuge at 1000g/20 min, at RT. Add 1N

HCl to filtrate, pH 4.0, centrifuge. Add ppt. w/ 1N

NaOH to pH 7.0.

Okezie and Bello, 1988

-**Isoelectric protein isolate**: extract defatted **winged**

bean flour (1:20) in H_2O at ph 10, 90 min/30^0C,

centrifuge at 10000 rpm/30 min. Precipitate at pH 4.0.

-**Neutralized protein isolate**: adjust to pH 12 at

30^0C/30 min, centrifuge. Adjust filtrate to pH 7.0.

Dried.

Tjahjadi et al., 1988

-**Albumin fraction**: extract **adzuki bean flour** (1:10) in

H_2O at 4^0C/24 h, centrifuge at 10000g/30 min. Dialyzed

filtrate in H_2O at 4^0C/24 h, freeze dried

-**Globulin fraction**: add 2% NaCl (1:10) to residues at

4^0C/24 h, centrifuge 10000g/30 min, dialyzed in H_2O at

4^0C/24 h, freeze dried.

-**Protein Isolate**: extract flour (1:10) in 0.01N NaOH,

at pH 8.5/2 h, centrifuge at 3000g/2 min. Add 1N HCl to

pH 4.0 to the filtrate, freeze dried the ppt.

Zarins et al., 1984

-**12S fraction**: extract **defatted cotton seed** flour w/

H_2O, 2 times, remove non storage protein. Add Tris-HCl

0.5M, NaCL 0.5 M, and 0.2 g/L NaN_3, adjust to ph 7.0.

Dilute supernatant w/ H_2O (1:10), centrifuge to collect

ppt. as 12S.

-**7S fraction**: Chilled filtrate to 0^0C, centrifuge at

0^0C. Dissolve ppt in 0.5 M Tris-0.5M NaCl, adjust to

pH 7.0, as 7S.

Bryant et al., 1988

-**Protein concentrate**: extract **defatted okra seed flour**

(1:20) in H_2O at pH 10 using 6N and 1 NaOH at 25^0C/60

min, centrifuge at 1293g/10 min, adjust filtrate to pH

7.0, freeze dried .

-**Protein isolate**: adjust supernatant to pH 4.5 w/ 6N

and 1N HCl, centrifuge at 1293g/20 min. Resuspend ppt.

in H_2O at pH 7.0, then freeze dried.

Bahnassey et al., 1986

-extract **legume flour (navy, pinto bean, lentils)**

(1:10) in 0.02% NaOH at RT for 1 h, centrifuge at 1500g

at RT/15 min, done 2 times. Adjust combined filtrate to

pH 4.5 w/ HCl concentrate, centrifuge at 1500g/15 min.

Wash ppt w/ H_2O, freeze dried.

Duszkiewicz-Reinhard et al., 1988

-extract **navy bean flour** (1:10) in 0.02N NaOH at RT/1

h, centrifuge, done 2 times. Add HCl concentrate to

filtrate (pH 4.5), centrifuge at 1500g/15 min. Wash

ppt. w/ H_2O, freeze dried.

Sathe et al., 1982

-extract **lupin seed flour** (1:5) in 0.02N NaOH at

21^0C/16 h, centrifuge at 10000g at 4^0C/30 min, done 2

times. Dialyzed combine filtrate against H_2O for 72 h

changes H_2O 6 times. Freeze dehydrated.

Sathe et al., 1981

> -extract **Northern bean flour** (1:10) in 0.5% Na_2CO_3 at
> 4^0C/24 h, centrifuge at 10000g/30 min. Dialyzed
> filtrate against H_2O for 48 h, change H_2O 6 times.
> Lyophilized.

Mustafa et al., 1986

> -extract **cowpea flour** (1:10) in phosphate buffer, pH
> 6.8, let stand for 20 h in refrigerator (2^0C). Blend in
> Waring blender at medium speed for 15 min, done 2
> times. Filter with cheese cloth. Centrifuge combined
> filtrate at 1000 rpm/1 min to ppt. starch. Add filtrate
> w/ 5% HCl to pH 4.5, let stand in refrigerator for 60
> min. Centrifuge and wash w/ H_2O. Vacuum dried the ppt.
> at 50-60^0C/5-7 h.

Tzeng et al., 1988

> -extract **rapeseed flour** (1:18) in 1% sodiun
> hexametaphosphate solution, pH 7.0. Stir in RT/30 min,
> adjust to pH 7.0 w/ 1-6N HCl or NaOH. Vacuum filtered
> to recover extract, put to ultrafiltration, ion
> exchange, then dried.

Appendix 2. Detail explanation on materials and methods used
in the experiment

Materials. Whole yellow peas (*Pisum sativum* L.
Miranda) of products sample grade US. No. 1 (based on USDA
Standards) were grown in 1989 and provided by International
Seeds Inc., Halsey, OR. Thirty-five kilograms of yellow peas
were ground into medium particles using Thomas Wiley Lab.
Mill Model 4, in Crop Science Dept., OSU, OR. Yellow pea
flour was weighed into ziploc-plastic bag for 100 g each,
then stored in tightly covered plastic bucket in cold room
(3^0C) until needed. Chemicals for protein extraction and
nitrogen determination, including liquid nitrogen, $CaCl_2$
$2H_2O$(Chem. MFG. Corp., Gardena, CA), $MgSO_4$ anhydrous (EM
Industries Inc., Cherry Hill, NJ) and other general reagents
were purchased from OSU Chemical Store (Corvallis, OR).
Acrylamide, bis-acrylamide, AMPS, TEMED, Na-lauryl sulfate,
bromophenol blue, 2-mercapto ethanol, and trizma base were
obtained from Sigma Chem. Co. (St. Louis, MO). Coomassie
blue R-250 was bought from JT. Baker Inc. (Phillipsburg,
NJ). The slab gel electrophoresis unit, SE 250 Mighty Small
II, was ordered from Hoefer Scientific Instruments (San
Francisco, CA). Other equipments and apparatus were provided
by Nutrition and Food Management Dept., OSU (Corvallis, OR).
Black and white prints and color slides were done in
Communication and Media Center Dept., OSU (Corvallis,OR).

Methods. <u>Protein Extraction</u>. Proteins of yellow peas were extracted according to the temperature, pH level, and coagulation treatments. Protein extraction procedure is reported in Figure 1. For each extraction, 100 g of pea flour was blended for 10 sec at low speed with 600 mL redistilled water in Waring Commercial Blender, and continued liquefying for an additional 1 min. The slurry was transferred and brought to 1 L with redistilled water after pH adjustment. The slurry was shaken in Gyrotory-shaker (New Brunswick Scientific Co., NJ), at speed 2.5 for 30 min with controlled temperature 25^0C or above, depending on the treatments assigned. Low temperature extractions (less than 25^0C) were done in low temperature incubator (Precision Scientific with Freas 815 temperature regulator) using a magnetic stirrer. Samples were then centrifuged (IEC-International Centrifuge, International Equipment Co., Needham, Mass.) at a speed of 15 (1800 rpm) for 10 min at room temperature (25 ± 2^0C). Collected protein was adjusted to pH 8 with 2N HCl or 2N NaOH then drop-frozen into liquid nitrogen and stored ("Revco" ultra low freezer, Revco Inc., West Columbia, S.C.) at -55^0C until it was freeze-vacuum dried (VirTis, Consol 4.5 Model; shelf temperature= 40^0C, vacuum pressure= 50 mT).

Yields were calculated from freeze-dried products. Each yield was powdered in a Waring Commercial Blender for 10 sec. Protein samples were kept in glass bottles and

refrigerated (3⁰C) until needed for protein
characterizations.

Calcium determination was done by ashing 2 g of
proteinate at 525°C for 24 h, dissolved in 3 mL of 3N HCl
solution and made to 25 mL volume with redistilled water.
The solution was analyzed using a Perkin Elmer 2380 atomic
absorption spectrometer.

Electrophoresis. Electrophoresis of the proteinate used
sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE)
according to the procedure of Laemmli (1970), using a
protein concentration of 1.25 mg/mL, with 20 μL sample
solution per well. Protein markers or molecular weight
standard of MW-SDS-70L (Sigma Chem. Co., St.Louis, MO) were
used with bromophenol blue as the tracking dye.

SDS-PAGE for all samples was done with and without
addition of 2-mercapto ethanol (2-ME) as a reducing agent,
using 13% and 12% acrylamide gels, respectively.
Electrophoresi were run at constant current, 40 ma or 35 ma
per 2 gels for unreduced or reduced samples, respectively.
Staining was done in 0.125% Coomassie brilliant blue R-250,
50% methanol, and 10% acetic acid, for 4 h. Destaining was
done in 50% methanol, 10% acetic acid, for 2 h, then
continued destaining in 5% methanol and 7% acetic acid, for
6 h. All assays were done at 25±2 ⁰C. Molecular weight of

protein subunits were estimated from the plot of log MW
versus the ratio of the distance traveled in comparison with
the tracking dye. The MWs were expressed in kilo Dalton (kD)
which is equal to 1.661×10^{-21} g. Gels were stored in 7%
glacial acetic acid solution until photographs were taken.

Nitrogen Solubility. Nitrogen solubility index (NSI) of
1% (w/v) proteinate solution was determined based on the
method of Thompson (1977). Prepare protein solution (0.01
g/mL H_2O) by stirring for 10 min, at room temperature. Take
7.5 mL protein solution then adjusted to 10 mL with H_2O, as
total aliquot, take duplicate of 1 mL and put into Kjeldahl
flasks. Prepare 11 centrifuge tubes filled with 7.5 mL
protein solution and adjust to pH 2-12 using 2N HCl or 2N
NaOH. Bring the volume to 10 mL with H_2O then stir for 3
min. Centrifuge those adjusted samples at 3000 rpm for 10
min. Take duplicate of 1 mL supernatant from each tube and
put into Kjeldahl flasks. All those samples are subject to
nitrogen determination (micro Kjeldahl, AOAC 1990). Nitrogen
solubility index is calculated as follow:

$$NSI = \frac{mg\ N/mL\ filtrate}{mg\ N/mL\ total\ aliquot} \times 100$$

Least gelation concentration (LGC). All samples from 13
different extractions (Soetrisno and Holmes, 1991) were
tested for LGC. Modified method of Tjahjadi et al. (1988)

was used. In the previous trial, protein concentrations used were 20, 18, 16, and 14% for AP, and 15, 13, 11, and 9% for MAP or CAP. The sample solutions were kept on top of boiling water bath for 30 min, cooled in the ice bath for 5 min and continued cooling in the refrigerator for 4 h. The LGC was determined as the lowest concentration of the proteinate that did not fall or run when test tube was inverted.

Fat adsorption (FA). All samples were tested for their FA values using method of Tjahjadi et al. (1988). Each sample was weighed 0.5 g and put into known weight of centrifuge tube. Add 3 mL oil while stirring on the vortex mixer (speed 4 of 1-10, VWR, Scientific Industries, Inc. Bohemia, NY), for 1 min. Let stand for 30 min $(25 + 2^0C)$, centrifuged at 3000 rpm for 25 min (IEC-International Centrifuge, International Equipment Co., Needham, Mass.). Removed the separated oil then inverted tube for 25 min (25 ± 2^0C) to drain unbound oil. Weigh the tube containing oiled sample, and corrected the weight for tube weight. Calculate the FA as:

$$\% \text{ FA} = \frac{(\text{reweighed sample - initial weight})}{\text{sample weight}} \times 100$$

Emulsion capacity (EC). The method was modified from Okezie and Bello (1988), using 0.5 g proteinate was dissolved in 12.5 mL 3% NaCL, homogenized in VirTis

homogenizer (speed 30 of 0-50, Research Equipment, Gardner, NY) for 30 sec. Add 30 mL oil while mixing (speed 10 mL/min), continue mixing for 30 sec. Transferred the emulsion into 50 mL centrifuge tube, kept in water bath (80^0C, 15 min), then centrifuged at 3000 rpm for 30 min. Record the volume of oil separated, and calculate EC as:

$$EC = \frac{\text{Vol. oil emulsified}}{\text{g sample}}$$

Appendix 3. Data on yield (%) of protein extraction from
        yellow pea flour

No.	^{0}C	pH	Extraction types		
			Acid	MgSO$_4$	CaCl$_2$
1	10	9.0	16.2	13.8	14.6
2	15	8.0	18.3	11.8	14.0
3	15	10.0	15.0	15.3	16.8
4	25	7.14	17.0	17.0	14.1
5	25	9.0	21.0	13.2	14.0
6	25	9.0	21.7	14.8	15.6
7	25	9.0	19.0	14.4	15.2
8	25	9.0	19.8	14.6	15.0
9	25	9.0	19.4	14.6	15.3
10	25	10.86	15.9	15.9	18.6
11	35	8.00	13.0	14.0	14.4
12	35	10.00	19.6	16.2	17.7
13	40	9.00	18.1	15.3	15.1

Appendix 4. Data on nitrogen content (%) of proteinates
extracted from yellow pea flour

No.	^0C	pH	Extraction types		
			Acid	$MgSO_4$	$CaCl_2$
1	10	9.00	12.10	12.70	11.66
2	15	8.00	12.48	12.30	11.84
3	15	10.00	12.14	12.35	12.55
4	25	7.14	11.90	12.42	11.12
5	25	9.00	11.92	12.77	11.86
6	25	9.00	11.77	12.74	11.76
7	25	9.00	12.03	12.01	11.72
8	25	9.00	11.93	11.68	11.38
9	25	9.00	11.65	11.25	11.90
10	25	10.86	11.11	11.70	11.42
11	35	8.00	11.82	12.21	11.30
12	35	10.00	12.13	11.11	11.32
13	40	9.00	11.79	11.70	11.35

Appendix 5. Data on fat adsorption (%) of proteinates
extracted from yellow pea flour

No.	^{0}C	pH	Extraction types		
			Acid	MgSO$_4$	CaCl$_2$
1	10	9.00	330.4	328.2	474.4
2	15	8.00	353.7	307.7	473.0
3	15	10.00	468.6	513.0	464.6
4	25	7.14	457.0	418.5	446.1
5	25	9.00	579.1	465.4	434.1
6	25	9.00	477.3	480.4	449.3
7	25	9.00	534.9	422.7	399.3
8	25	9.00	461.6	393.5	406.6
9	25	9.00	553.3	463.7	487.7
10	25	10.86	482.7	447.5	454.7
11	35	8.00	517.6	465.4	458.6
12	35	10.00	501.2	422.2	300.9
13	40	9.00	478.9	343.3	299.6

Appendix 6. Data on least gelation concentration (%) of
proteinates extracted from yellow pea flour

No.	^{0}C	pH	Extraction types		
			Acid	MgSO$_4$	CaCl$_2$
1	10	9.00	16	15	15
2	15	8.00	14	13	15
3	15	10.00	18	15	15
4	25	7.14	17	15	15
5	25	9.00	18	15	15
6	25	9.00	18	15	15
7	25	9.00	18	15	15
8	25	9.00	19	15	15
9	25	9.00	18	15	15
10	25	10.86	17	15	15
11	35	8.00	20	15	15
12	35	10.00	16	15	15
13	40	9.00	19	15	15

Appendix 7. Data on nitrogen solubility index (%) of acid proteinates
extracted from yellow pea flour

Extraction		pH solubilization										
°C	pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
10	9.00	64.4	64.4	27.1	0.0	52.2	59.3	64.4	71.2	74.5	81.3	98.3
15	8.00	78.1	78.1	20.4	5.3	49.6	71.3	76.1	80.8	83.4	89.5	96.8
15	10.00	45.9	50.6	0.0	0.0	52.8	52.8	55.2	57.4	62.0	72.4	80.4
25	7.14	93.0	73.0	5.4	0.0	70.3	82.4	93.2	94.6	100.0	100.0	96.0
25	9.00	75.9	79.3	68.9	0.0	67.2	75.9	72.4	81.0	87.9	100.0	100.0
25	9.00	72.0	63.2	5.9	0.0	61.8	67.6	85.3	88.2	92.6	100.0	100.0
25	9.00	74.9	72.0	0.0	5.9	76.5	76.5	85.3	88.2	94.1	98.5	100.0
25	9.00	74.4	74.4	56.4	0.0	0.0	80.0	76.9	82.0	94.9	96.1	97.4
25	9.00	87.0	87.0	60.9	0.0	55.1	78.3	81.0	85.8	85.8	88.4	91.0
25	10.86	76.0	76.0	64.0	12.0	8.0	68.0	72.0	76.0	100.0	100.0	100.0
35	8.00	68.0	65.8	18.9	0.0	8.0	62.0	71.3	73.4	72.0	97.0	97.2
35	10.00	61.8	69.4	38.9	0.0	40.3	66.6	68.0	75.7	75.7	94.4	98.6
40	9.00	67.2	65.5	50.0	0.0	10.3	72.5	72.5	77.6	75.9	82.8	94.8

Appendix 8. Data on nitrogen solubility index (%) of magnesium proteinate
extracted from yellow pea flour

Extraction		pH solubilization										
⁰C	pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
10	9.00	61.0	56.1	23.2	0.0	0.0	4.9	42.7	53.6	62.2	80.4	96.3
15	8.00	68.9	55.9	5.2	5.2	0.0	18.2	31.2	61.0	66.2	74.0	77.9
15	10.00	46.4	50.3	31.2	0.0	3.0	7.7	18.8	23.8	37.2	45.5	73.4
25	7.14	66.9	75.8	43.7	0.0	0.0	11.6	19.1	43.6	44.4	89.9	95.6
25	9.00	59.0	55.1	35.9	0.0	0.0	7.7	58.9	66.7	60.2	76.9	92.3
25	9.00	73.1	61.6	51.3	5.1	0.0	7.7	25.6	57.7	62.8	71.8	78.2
25	9.00	66.7	66.7	12.1	0.0	6.1	9.1	30.3	42.4	51.5	63.6	97.0
25	9.00	60.5	64.7	47.4	0.0	0.0	8.8	8.8	25.4	57.4	76.5	100.0
25	9.00	67.9	46.0	21.2	4.5	0.0	6.8	8.2	48.0	59.4	76.8	83.6
25	10.86	47.8	37.6	8.7	0.0	8.7	14.5	14.5	29.0	44.9	91.2	97.0
35	8.00	61.6	54.8	57.5	0.0	0.0	5.5	11.0	56.2	57.5	71.2	89.0
35	10.00	38.1	33.4	11.9	0.0	4.8	4.8	14.3	17.9	45.3	73.7	92.9
40	9.00	56.0	48.4	13.6	0.0	0.0	0.0	16.6	45.4	53.0	71.2	93.9

Appendix 9. Data on nitrogen solubility index (%) of calcium proteinate
extracted from yellow pea flour

Extraction		pH solubilization										
^{0}C	pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
10	9.00	39.4	28.6	7.2	0.0	0.0	7.2	7.2	10.7	21.4	28.6	50.0
15	8.00	66.7	59.3	22.2	11.1	0.0	11.1	7.4	11.1	14.8	59.2	81.5
15	10.00	50.0	33.4	17.4	0.0	3.6	2.9	5.8	13.1	27.6	45.7	76.8
25	7.14	57.8	34.4	0.0	0.0	0.0	6.3	15.6	34.4	50.0	70.3	79.7
25	9.00	53.9	47.7	20.9	0.0	0.0	0.0	9.0	9.0	29.8	50.7	59.7
25	9.00	51.1	40.4	23.4	0.0	0.0	12.8	10.6	12.7	19.2	48.9	53.2
25	9.00	55.9	56.7	35.3	0.0	0.0	11.7	8.3	16.7	29.4	58.8	82.4
25	9.00	51.6	45.2	23.5	0.0	0.0	8.8	6.5	12.9	19.4	41.2	64.5
25	9.00	52.9	32.4	5.9	0.0	8.8	8.8	11.8	17.6	20.6	78.0	91.2
25	10.86	48.6	32.0	19.5	11.2	0.0	8.3	8.4	11.1	30.5	40.3	75.0
35	8.00	54.1	39.6	12.5	0.0	0.0	8.4	8.4	8.4	20.8	45.9	62.5
35	10.00	40.3	12.0	0.0	0.0	0.0	0.0	6.0	8.9	22.0	41.8	86.6
40	9.00	41.6	29.2	8.3	0.0	5.6	5.6	11.3	8.3	30.5	54.2	64.4

Appendix 10. Data on emulsion stability (mL retained oil/g proteinate) after time

Extraction		AP[a]				MAP[b] Time (h)				CAP[c]			
[0]C	pH	0.0	0.5	2.0	6.0	0.0	0.5	2.0	6.0	0.0	0.5	2.0	6.0
10	9.00	50	50	50	50	50	50	50	30	50	50	50	49
15	8.00	50	50	50	50	50	50	50	28	50	50	50	46
15	10.00	50	50	50	50	50	50	36	29	50	50	42	24
25	7.14	50	50	50	50	50	50	50	23	50	50	50	50
25	9.00	50	50	50	50	50	50	42	26	50	50	50	46
25	9.00	50	50	50	50	50	50	40	30	50	50	50	48
25	9.00	50	50	50	50	50	50	50	44	50	50	50	48
25	9.00	50	50	50	50	50	50	50	50	50	50	50	50
25	9.00	50	50	50	50	50	50	50	24	50	50	50	38
25	10.86	50	50	50	50	50	50	50	50	50	50	50	50
35	8.00	50	50	50	50	50	50	50	36	50	50	50	47
35	10.00	50	50	50	50	50	50	48	44	50	50	48	48
40	9.00	50	50	50	50	50	50	50	32	50	50	50	50

[a]AP is acid proteinate
[b]MAP is magnesium proteinate
[c]CAP is calcium proteinate

Appendix 11. Data on emulsion stability (mL retained water/g proteinate) after time

Extraction		AP[a]				MAP[b] Time (h)				CAP[c]			
^{0}C	pH	0.0	0.5	2.0	6.0	0.0	0.5	2.0	6.0	0.0	0.5	2.0	6.0
10	9.00	25	25	25	25	25	25	25	25	25	25	25	25
15	8.00	25	25	25	25	25	25	25	25	25	25	25	25
15	10.00	25	25	25	25	25	25	25	25	25	25	25	22
25	7.14	25	25	25	25	25	25	25	25	25	25	25	23
25	9.00	25	25	25	25	25	25	25	25	25	25	25	25
25	9.00	25	25	25	25	25	25	25	25	25	25	25	25
25	9.00	25	25	25	25	25	25	25	24	25	25	25	25
25	9.00	25	25	25	25	25	25	25	25	25	25	25	25
25	9.00	25	25	25	25	25	25	25	24	25	25	25	25
25	10.86	25	25	25	25	25	24	21	19	25	24	21	15
35	8.00	25	25	25	25	25	25	25	25	25	25	25	25
35	10.00	25	25	25	25	25	25	23	20	25	25	23	17
40	9.00	25	25	25	25	25	24	25	25	25	25	25	25

[a]AP is acid proteinate
[b]MAP is magnesium proteinate
[c]CAP is calcium proteinate

Appendix 12. Data on emulsion capacity (mL oil/g proteinate) of acid (AP), magnesium (MAP), and calcium (CAP) proteinates

Extraction		AP	MAP	CAP
^{0}C	pH			
10	9.00	58.0	59.8	4.0
15	8.00	52.0	50.0	6.0
15	10.00	60.0	2.0	0.0
25	7.14	58.0	58.0	60.0
25	9.00	60.0	60.0	56.0
25	9.00	60.0	60.0	59.6
25	9.00	60.0	60.0	60.0
25	9.00	58.0	56.0	59.6
25	9.00	60.0	56.0	60.0
25	10.86	60.0	10.0	2.0
35	8.00	59.8	59.8	2.0
35	10.00	60.0	2.0	0.0
40	9.00	60.0	34.0	60.0

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