1990

Functional properties and in-vitro digestibility of green gram protein products

Manjulatha Mokkarala
San Jose State University

Follow this and additional works at: https://scholarworks.sjsu.edu/etd_theses

Recommended Citation

Mokkarala, Manjulatha, "Functional properties and in-vitro digestibility of green gram protein products" (1990). *Master's Theses*. 3332.
DOI: <https://doi.org/10.31979/etd.3s8c-fcwp>
https://scholarworks.sjsu.edu/etd_theses/3332

This Thesis is brought to you for free and open access by the Master's Theses and Graduate Research at SJSU ScholarWorks. It has been accepted for inclusion in Master's Theses by an authorized administrator of SJSU ScholarWorks. For more information, please contact scholarworks@sjsu.edu.

INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 1341683

**Functional properties and *in-vitro* digestability of green gram
protein products**

Mokkarala, Manjulatha, M.S.

San Jose State University, 1990

U·M·I

**300 N. Zeeb Rd.
Ann Arbor, MI 48106**

FUNCTIONAL PROPERTIES AND IN-VITRO DIGESTIBILITY OF
GREEN GRAM PROTEIN PRODUCTS

A Thesis

Presented to

The Faculty of the Department of Nutrition and Food
Science

San Jose State University

In Partial Fulfilment
of the Requirements for the Degree
Master of Science
in Nutritional Science

By

Manjulatha Mokkarala

August, 1990

APPROVED FOR THE DEPARTMENT OF
NUTRITION AND FOOD SCIENCE

Dr. Panfilo Belo

Dr. Elveda Smith

Dr. Miriam Saltmarch

APPROVED FOR THE UNIVERSITY

ABSTRACT

FUNCTIONAL PROPERTIES AND IN-VITRO DIGESTIBILITY OF GREEN GRAM PROTEIN PRODUCTS

by Manjulatha Mokkarala

Ground dehulled green gram flakes were subjected to batchwise hexane extraction, and the resulting defatted meal was used to prepare flour, concentrate, and isolate. The protein content, on a dry basis, of the defatted flour, concentrate, and isolate were 25.3, 73.0, and 92.9%, respectively.

The percent protein solubility of the three products was maximum at pH 9.0 and minimum at pH 4.5. The flour showed the highest protein solubility, and the isolate showed the lowest. The emulsifying and foaming properties of the products were higher at pH 9.0 and lower at pH 4.5. At both pH conditions the isolate exhibited the highest emulsifying capacity. There was no significant difference in the percent volume increase of foam produced by the flour, concentrate, and isolate. However, the isolate showed the lowest percent drip. A significant difference was found between the in-vitro protein digestibility values for the isolate, concentrate, and flour.

ACKNOWLEDGEMENTS

The author gratefully acknowledges: Dr. Panfilo Belo, my graduate advisor, for his time, patience, and advice throughout all phases of this research; Dr. Elveda Smith and Dr. Miriam Saltmarch for guidance and assistance with the organization of the manuscript.

Dedication

This thesis is dedicated to my husband, Vigg
Mokkarala, whose encouragement and support made it possible
for me to achieve this academic goal.

PREFACE

The following is a publication style thesis. The second chapter is written in journal format according to the January 1990 guidelines and will be submitted to the Journal of Agricultural and Food Chemistry. Chapters 1 and 3 are written according to the guidelines outlined in the Publication Manual of the American Psychological Association, 3rd. edition, 1986.

Table of Contents

	Page
List of Tables	viii
List of Figures	x
 CHAPTER	
1 INTRODUCTION AND REVIEW OF LITERATURE	1
Introduction	1
Objectives	2
Significance of the Study	3
Review of Literature	4
Green Gram	4
Composition	4
Proteins	6
Functionality of Proteins	8
Solubility	11
Emulsification	13
Foaming	16
Protein Products from Plant Seeds	18
In-vitro Protein Digestibility	19
2 JOURNAL ARTICLE	21
Author's Title Page	22
Abstract	23
Introduction	24
Materials and Methods	25
Sample Preparation	25

Experimental Design	27
Proximate Analysis	28
Solubility Determination	28
Emulsifying Properties	28
Foaming Properties	29
In-vitro Protein Digestibility	30
Analysis of Data	31
Results and Discussion	31
Solubility	32
Emulsification	35
Foaming	37
In-vitro Protein Digestibility	38
Conclusions	39
Literature Cited	49
Funding Note	51
3 SUMMARY AND RECOMMENDATIONS	52
Summary	52
Recommendations	55
REFERENCES	57

List of Tables

Table		Page
I.	Composition of Mature Green Gram Seeds Compared with other Legumes (in 100 g Edible Portion)	5
II.	Essential Amino Acid Composition of Green Gram Proteins (g/16 g N) Compared to Whole Chicken Egg Protein	7
III.	Functional Properties of Proteins and Their Mode of Action in Actual Food Systems	10
1.	Proximate Analysis of the Flour, Concentrate, and Isolate from Defatted Dehulled Green Gram on Wet Basis	44
2.	Comparison of Solubility in 1% (w/v) Aqueous Dispersions of Green Gram Flour, Concentrate, and Isolate Containing 0.1 M NaCl at Room Temperature (26° C)	45
3.	Effect of pH on Emulsifying Properties in 8% (w/v) Aqueous Dispersions of Green Gram Protein Products at Room Temperature (26° C) and 0.1 M NaCl	46
4.	Effect of pH on Foaming Properties in 1% (w/v) Aqueous Dispersions of Green Gram Protein Products at Room Temperature (26° C) and 0.1 M NaCl	47

List of Tables

Table	Page
5. In-vitro Protein Digestibility (IVPD) of Green Gram Protein Products	48

List of Figures

Figure	Page
1. Preparation of Green Gram Protein	
Isolate from Defatted Flour	42
2. Effect of pH on Solubility of 1% (w/v)	
Aqueous Dispersions of Green Gram	
Protein Products Containing 0.1 M NaCl	
at Room Temperature	43

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

The importance of using plant proteins in areas where protein-calorie malnutrition (PCM) exists is well recognized (Salunkhe, 1982). Legumes constitute a staple part of the dietary protein of a large segment of the world population especially where animal proteins are scarce or are not being utilized (West, 1984). The increasing cost of animal proteins has prompted food technologists, nutritionists, and biochemists to find alternative sources of proteins, particularly plant proteins (Rakosky, 1987).

Green gram (Vigna radiata [L] Wilczek) is among the most commonly used legumes in Africa and Asia (Mesallam & Hamza, 1987). In some regions of India, the legume constitutes a major portion of the dietary protein. The plant grows on all types of soils and is a short duration crop. It grows successfully under the most adverse arid and semi-arid conditions where other seed crops fail (Adsule, Kadam, & Salunkhe, 1986). The green gram plant produces seeds which contain, on average, 22.5% by weight protein. Since the plant yields 400 to 600 kg/hectare, Krishnamurthy and Rama Rao (1976) suggested that green gram is a cheap source of protein.

Although the potential of green gram as a protein rich source has stimulated research interest in recent years, the database on this legume needs to be expanded to facilitate efficient utilization of its protein products. Since green gram seeds are widely used as a protein source (Mesallam & Hamza, 1987), it is important to fully understand the functional properties of the protein constituents. Based on the development of soy protein products, ingredient proteins should have acceptable intrinsic properties such as flavor, texture, color, good nutritional value, and requisite functional properties for a variety of intended applications (Hermannson, 1979; Kinsella, 1979). Properties such as solubility, viscosity, texture, emulsion, foam, and gel characteristics are essential in the technology and application of green gram proteins.

Objectives

The objectives of this study were: 1) to prepare green gram flour, concentrate, and isolate; 2) to investigate the functional properties such as solubility, emulsification, and foaming properties of the flour, concentrate, and isolate; and 3) to determine the in-vitro protein digestibility (IVPD) of green gram protein products.

Significance of the Study

Food scientists and nutritionists in recent years have recognized the value of plant proteins in supplying the protein needs in developing countries where PCM prevails. Green gram is one of the few legumes which is well adapted to grow in areas where foods are poor in protein (Adsule, Kadam, & Salunkhe, 1982). Despite the increased interest in green gram in recent years, the database is still limited. With continued research, green gram may become a significant food crop in supplying the protein needs of underdeveloped countries.

The functionality of proteins determines their application and use in various food products. The basic information obtained in the study is essential in developing methods for processing green gram into protein products. Basic information on physical and chemical properties and digestibility of green gram proteins is limited and fragmented, and gaps in information need to be filled. This study was designed to investigate the solubility properties of green gram flour, concentrate, and isolate at various pH conditions; to study the emulsification and foaming properties at the pH at which the solubility is highest and lowest; and, finally, to investigate the IVPD of these green gram protein products.

Review of Literature

Green Gram

Green gram grows throughout Southeast Asia, Central Africa, the warmer parts of China, and in the United States. In India, this seed ranks third in production next to chick pea and pigeonpea and is extensively grown on all types of soils and under varying climatic conditions. It is a short duration crop and has wide adaptability. Green gram grows successfully under most adverse arid and semi-arid conditions where other seed crops always fail (Adsule, Kadam, & Salunkhe, 1986). The seeds are green or brown colored with a flat hilum.

Composition

Data in Table I compare the proximate composition and mineral and vitamin contents of green gram with the soy bean, cowpea, and chick pea. Cowpea and chick pea are legumes that are commonly grown in the underdeveloped and developing countries. The proximate composition of mature green gram seeds is similar to that of cowpea and chick pea, but it is different from soy bean. The crude protein content of green gram is comparable to the cowpea but is slightly higher than that of the chick pea and lower than that of the soy bean. The fat content of green gram is similar to the cowpea, is about 17 times lower than that of the soy bean, and is approximately 6 times lower than that

Table I

Composition of Mature Green Gram Seeds Compared With Other Legumes (in 100 g Edible Portion)

	Green gram	Cowpea	Chick pea	Soy bean
<u>Proximate</u>				
<u>Composition</u>				
Moisture (g)	10.60	11.50	11.00	10.20
Fat (g)	1.20	1.60	5.60	17.70
Crude Protein (g)	22.90	22.70	19.40	35.10
Carbohydrates (g)	61.80	61.00	60.90	32.00
Crude Fiber (g)	4.40	4.20	2.50	4.20
<u>Minerals</u>				
Calcium (mg)	105.00	110.00	114.00	226.00
Potassium (mg)	1132.00	688.00	1006.00	1504.00
Iron (mg)	7.00	6.50	2.20	8.50
<u>Vitamins</u>				
Thianine (mg)	0.53	0.59	0.46	0.66
Riboflavin (mg)	0.26	0.22	0.20	0.22

Note. From "Chemistry and Technology of Green Gram (Vigna radiata [L] Wilczek)" by R.N. Adsule, S.S. Kadam, and D.K. Salunkhe, 1986, CRC Critical Reviews in Food Science and Nutrition, 25, p.75.

of the chick pea. Except for potassium, the mineral content of green gram is comparable to chick pea and cowpea and is lower than that of the soy bean. The potassium content of the green gram seed is considerably higher than the cowpea and chick pea and is lower than the soy bean. Ascorbic acid content of the green gram seed is markedly higher than the other three seeds, whereas the content of other vitamins is comparable among the four seeds.

Compared to cowpea and chick pea, proteins in green gram have a more balanced amino acid composition (Adsule, Kadam, & Salunkhe, 1986). Data in Table II show the essential amino acid composition of green gram proteins compared to the Food and Agricultural Organization (FAO) whole chicken egg reference protein. Methionine is the first limiting amino acid, and tryptophan is the second limiting amino acid. Due to the relatively high content of lysine in green gram, it is used in complimenting cereal diets which are low in lysine (Chatterjee & Abrol, 1975).

Proteins

The storage proteins in green gram have been studied and isolated. Derbyshire, Wright, and Boulter (1976) identified two fractions of globulins in green gram. The legumin fraction is an 11s component dissociated into three sub-units with molecular weights of 37,000, 34,000, and

Table II

Essential Amino Acid Composition of Green Gram Proteins
(g/16 g N) Compared to Whole Chicken Egg Protein

Amino acid	Green gram proteins	Whole chicken egg protein
Lysine	6.9	7.2
Threonine	3.0	5.2
Valine	6.4	7.4
Leucine	7.9	7.8
Isoleucine	5.4	6.8
Methionine	1.5	3.4
Tryptophan	0.5	1.5
Phenylalanine	5.7	5.8
Arginine	6.7	6.7
Histidine	2.7	2.4

Note. From "Chemistry and Technology of Green Gram (Vigna radiata [L] Wilczek)" by R.N. Adsule, S.S. Kadam, and D.K. Salunkhe, 1986, CRC Critical Reviews in Food Science and Nutrition, 25, p.79.

20,000. Vicilin has a sedimentation coefficient of 8S and contains four different protein sub-units with molecular weights of 63,000, 50,000, 29,000, and 24,000 (Derbyshire, Wright, & Boulter, 1976).

Green gram proteins are nutritionally inferior to whole egg proteins. The digestibility coefficient (DC), biological value (BV), protein efficiency ratio (PER), and net protein utilization (NPU) of green gram proteins are 81.0, 70.0, 2.1, and 46.0, respectively (Hanumantha & Subramanian, 1970). There is a significant correlation ($r = 0.97$) between BV and the total sulfur containing amino acids indicating that methionine, cysteine, and cystine are the first limiting amino acids in green gram (Coffman & Garcia, 1979). The lower digestibility of green gram proteins is attributed to the presence of antinutritional factors such as protease inhibitors (Mesallam & Hamza, 1987). According to Adsule, Kadam, and Salunkhe (1986), reduction or elimination of the antinutritional factors and improvement of digestibility and nutritive value would make the green gram more acceptable as an inexpensive nutritious food ingredient.

Functionality of Proteins

The functional properties of proteins are important in determining their application in food systems in which they are used as ingredients (Kinsella, 1979). In exploring new

sources of food proteins, it is necessary to study the functional properties to assess potential uses in foods (Huang & Kinsella, 1987). Functional properties determine the manner and quantity in which proteins can be incorporated into various food systems (Kramer & Kwee, 1977).

The physical and chemical properties of proteins determine the functional properties (Mattil, 1971). Functional properties (e.g., solubility and foaming) are determined by a balance between forces involving protein-protein, protein-solvent, and protein-solute interactions (Hermansson, 1979). These interactions, in turn, are influenced by the nature of the protein, the presence of other ingredients in a food system to which the protein ingredient is added, the manufacturing conditions, and the environmental factors (Huang & Kinsella, 1987). In testing the functionality of proteins, it is necessary to simulate the environmental conditions (e.g., pH, temperature, and ionic strength) to which the proteins are exposed in food systems (Johnson, 1970).

Functional properties of proteins and their mode of action in various food systems are shown in Table III. The desired functional properties of proteins vary from one food system to another (Hermansson, 1979). Solubility is the most important functional property of proteins intended

Table III

Functional Properties of Proteins and Their Mode of Action
in Actual Food Systems

Functional property	Mode of action	Food system
Solubility	protein solvation, pH dependent	beverages
Water absorption	hydrogen-bonding of water, entrapment of water, no drip	meats, sausages, breads, cakes
Viscosity	thickening, water binding	soups, gravies
Gelation	protein matrix formation, and setting	meats, curds, cheeses
Emulsification	formation and stabilization of fat emulsions	sausages, bologne, soup, cakes
Foaming	forms stable films to entrap gas	whipped toppings, chiffon desserts, angel cakes

Note: From "Functional Properties of Soy Beans" by J.E.
 Kinsella, 1979, Journal of American Oil Chemists' Society,
 56, p.245.

for beverages. Water absorption, cohesion-adhesion, elasticity, and fat absorption are important functional properties of proteins in the formulation of meats, sausages, and bakery products. Similarly, emulsification and viscosity are important in the formulation of sausages, soups, gravies, and cakes.

Solubility

Solubility is the most important functional property of proteins since it influences other functional properties such as emulsification, foaming, and viscosity (Hermansson, 1979; Mattil, 1971; McWatters et al., 1976). Protein solubility is commonly expressed as water-soluble nitrogen, nitrogen solubility index, water-soluble protein, or protein dispersibility index (Kinsella, 1979). Solubility of protein products depends upon the physicochemical state of the protein molecules, which are either favorably or adversely affected by heating, drying, and other processing treatments during manufacture and storage (Johnson, 1970).

Solubility of various legume seed proteins is affected by temperature, pH, and ionic strength (Cherry, McWatters, & Beuchat, 1979; Kinsella, 1979; Sosulski et al., 1976). Increasing temperature from 25° C to 60° C causes small increases in solubility of soybean protein isolates (Kinsella, 1979). However, heat treatment at or near 100° C generally reduces solubility due to protein denaturation.

In some cases, denaturation and solubility are not always correlated; high solubility data have been obtained from completely denatured proteins (Hermansson, 1979).

Soy proteins show the classical pH-solubility profile with an isoelectric point of pH 4.5 (Kinsella, 1979). According to Hermansson (1979), solubility of soy proteins decreases with an increase in pH from 2.0 to 4.5; from pH 4.5 to pH 9.0 or 10.0, solubility increases; and beyond pH 10.0, solubility decreases gradually. In general, the solubility of most plant proteins is lowest at the isoelectric point (pH 4.5) (Kinsella, 1979; Mattil, 1971; Wolf, 1970).

According to Kinsella (1979), the effect of sodium chloride (NaCl) on the solubility of a protein may be due to the high affinity of chloride ions to proteins. Chloride ions bind to the positively charged protein groups, especially in the acidic pH range, and enhance solubility by increasing the effect of electrostatic repulsion. Coffmann and Garcia (1979) studied the effect of pH and ionic strength on solubility of mung bean protein isolate and showed that the protein isolate was nearly 100% soluble at pH 10.5. The lowest solubility was recorded at pH 4.5 which is the isoelectric point. However, when the ionic strength of the dispersion increased from 0.1 M to 1.0 M NaCl, solubility of the protein increased.

Emulsification

An emulsion is a dispersion of liquid in liquid. In an oil-in-water emulsion, the continuous phase is water, and the dispersed phase is oil. The process of emulsification involves dispersing oil in a liquid medium and lowering the surface tension between the oil/water interface by surface active agents with the formation of smaller, uniformly distributed particles. Protein acts as an emulsifier by forming a film or membrane between the dispersed and continuous phases, thus stabilizing the emulsion. This property is generally expressed as emulsifying capacity and measures the maximum amount of oil that can be added to a fixed amount of protein dispersion without phase separation.

In general, the emulsifying property of proteins is influenced by solubility, the source of protein used, the difference in total protein composition and components other than proteins, the pH, the temperature, and the ionic strength of the dispersion medium. McWatters et al. (1976) found that proteins, with poor solubility properties under certain environmental conditions, have poor emulsifying properties because the protein is not free in solution to stabilize the oil/water interface. Inklaar and Fortuin (1969) observed that emulsion capacity (grams oil emulsified/grams protein) increased with an increase in

protein concentration. However, others cite evidence that there is no direct correlation between emulsification properties and aqueous solubility of proteins which could be, in part, due to the nature of the proteins (Aoki et al., 1980).

McWatters and Cherry (1977) found that soy bean flour suspended in water performed best at pH 6.2, whereas peanut, field pea, and pecan flours were best near pH 8.2. Flours of soy bean, peanut, field pea, and pecan have the poorest emulsification performance at pH 4.0, the approximate iso-electric point of many seed proteins (McWatters et al., 1976). Mesallam and Hamza (1987) reported similar findings for green gram protein concentrate in addition to the high emulsification capacity of the proteins at pH 10.0. Hutton and Campbell (1977) attributed this to the effect of pH on solubility.

The effect of temperature on the emulsifying property was determined with peanut protein by Cherry, McWatters, and Beuchat (1979). They found that moist heated peanut isolate had emulsion capacities similar to or better than a non-heated control. Salt, which adds to the ionic environment, reduces charge repulsion between proteins in an emulsion and, therefore, enhances hydrophilic association at the interface. When this occurs, emulsification performance increases (Kinsella, 1979).

Emulsification properties of proteins are important in the formulation of chopped comminuted meats, cake batters, coffee whiteners, milk, mayonnaise, salad dressings, and frozen desserts. For these products, differing emulsifying and stabilizing capacities of proteins are needed due to the varying composition and stresses (e.g., temperature and pH) to which these products are subjected (Kinsella, 1979).

At present, there is no standard method for the determination of emulsification properties. However, the methods used in determining the emulsification capacity of proteins essentially involve suspending a pre-determined amount of protein in water, solubilizing it by blending for 30 sec, and then adding oil at a specified rate until the emulsion breakpoint is reached as indicated by separation of oil and water into two layers. The amount of oil added is a measure of the emulsification property (Beuchat, 1977; McWatters & Cherry, 1977).

To describe the various consistencies of emulsions formed, an arbitrary classification was suggested by McWatters and Cherry (1977) based on actual viscosity measurements of the emulsions. When the viscosity of the emulsion is 160,000 centipoises (cps), the consistency of the emulsion formed is described as very thick and mayonnaise like; 112,320 cps, thick, mayonnaise like; 60,160 to 70,880 cps, semi thick, mayonnaise like; 35,680

to 47,680 cps, very thick, salad dressing like; 17,280 to 26,080 cps, semi thick, salad dressing like; and, 5,760 to 12,960 cps, thin, pourable suspension.

Foaming

Foaming properties are affected by the concentration of proteins in a solution, the type of protein, pH, temperature, ionic strength of the suspension, and the degree and duration of whipping. Eldridge, Hall, and Wolf (1963b) observed that a 10% protein suspension produced an extremely stiff foam compared to a 3% protein suspension. However, no significant difference in the percent volume increase was observed. Johnson (1970) found that soy protein isolates have superior foaming qualities than soy flour and concentrate. These differences were attributed to the high lipid content of the latter compared to the isolate. Lipids are detrimental to foam formation since they destabilize the protein films (Kinsella, 1979). Eldridge et al. (1963b) determined that the minimum foam expansion and stability for soy protein isolate ranged from pH 4 to 6, and the maximum foam expansion and stability were between pH 7 to 9. McWatters and Cherry (1977) showed that heating the mixture to 70° C or 80° C improved foaming properties of soy protein. However, Eldridge et al. (1963b) noted that a relatively stable foam can be obtained

without heat but that foam expansion and stability improved when heat is applied.

Coffmann and Garcia (1979) found that high-speed mixers yield greater percentage volume increases and slightly more stable foams within a given whipping time. They also showed that longer whipping time (10 min) gave not only higher specific volume but also a larger percent volume increase than 5 min whipping time but that the foam stability was not significantly higher. Increasing the salt concentration reduces the percentage of protein in soluble fraction and, therefore, depresses foam formation (Cherry, McWatters, & Beuchat, 1979). Increasing the protein concentration increases stability but decreases expansion (Eldridge, Hall, & Wolf, 1963a).

The foaming property of proteins has wide application in the food industry (Kitabatake & Doi, 1988). Foaming is an important property in cakes (angel, sponge), souffles, and whipped toppings (Table III, p.10). To produce a good quality foam, proteins should be soluble in the aqueous phase, concentrate at the interface, and unfold to form cohesive layers of protein around air droplets as the air droplets are formed (Kinsella, 1979).

There is no standard method to evaluate foaming characteristics. The basic steps involved in the determination of foam characteristics of proteins include

dispersing a specified amount of protein in an aqueous solution and mixing, blending, whipping, or shaking it for a period of time at a specified speed. This is followed by the determination of specific density and stability of the foam (Eldridge et al., 1963b; Lawhon & Cater, 1971; McWatters & Cherry, 1977). Current methods used differ both in the type of equipment and the duration of blending or whipping. For example, Lawhon and Cater (1971) used a shaker to develop foam; McWatters and Cherry (1977) used a blender; Eldridge et al. (1963b) used a mixer.

Protein Products from Plant Seeds

Legume protein products are commercially available in the form of flours and grits, concentrates, and isolates. These products are currently used in the preparation of a variety of foods due to their desirable functional properties. Legume flours and grits are the least refined forms with varying fat contents, particle sizes, and textures. Commercial defatted legume flours are prepared by grinding defatted legume flakes to 100 mesh or finer, whereas grits are coarser than 100 mesh. Maximum protein content of these materials range from 40 to 50% depending on the fat content (Wolf, 1970).

Protein concentrates are more refined than flours and grits and usually contain 50 to 75% of protein on a dry basis. These products are generally prepared from defatted

flakes or flour by removing the water-soluble polysaccharides, oligosaccharides, some minerals, and some minor components (Wolf, 1970).

Protein isolates are the most refined forms of plant protein products which generally contain 90% or more protein (Kinsella, 1979). They are prepared by removing the water-insoluble polysaccharides, as well as the oligosaccharides and other low molecular weight components that are not separated in the preparation of protein concentrates (Wolf, 1970). Mesallam and Hamza (1987) prepared green gram protein isolate by extracting dehulled green gram flour with 300 ml of 0.5 M NaCl solution at room temperature for 1 hr with stirring. The extract was centrifuged at 3000 rpm for 15 min. The supernatant was diluted with cold tap water (5:1 v/v) and centrifuged at 3000 rpm for 15 min. The precipitate was washed with distilled water, freeze dried, and stored. According to Mesallam and Hamza (1987), isolated proteins often improve the appearance and taste compared with the original meal and, thus, can be better utilized as nutritional and functional ingredients in food products (Sathe & Salunkhe, 1981).

In-vitro Protein Digestibility

Although the amino acid profile is important in evaluating the nutritive quality of a protein, the

digestibility of the protein is the primary determinant of the availability of its amino acids. The digestibility of a food protein may be obtained from rat bioassays, but it is an expensive and time-consuming procedure. Several in-vitro methods for the measurement of protein digestibility have been developed which are rapid, reliable, and less expensive (Hsu et al., 1977).

Akeson and Stahmann (1964) found that a pepsin-pancreatin enzyme system gave a reasonable assessment of protein digestibility. The enzyme system described by Akeson and Stahmann (1964) was re-investigated by Saunders et al. (1973) and showed an excellent correlation with in-vivo data ($r = 0.88$). Mesallam and Hamza (1987) studied the in-vitro digestibility of green gram protein isolate using proteolytic enzymes. The digestibility by pepsin followed by pancreatin was greater than with either pepsin or pancreatin alone. This was attributed to the natural sequence prevailing in the gastro-intestinal tract whereby proteins are first exposed to the action of pepsin which splits the large protein molecules into smaller fragments which, in turn, are more easily attacked by pancreatin proteolytic enzyme.

CHAPTER 2
JOURNAL ARTICLE

FUNCTIONAL PROPERTIES AND IN VITRO DIGESTIBILITY
OF GREEN GRAM PROTEIN PRODUCTS

Manjulatha Mokkarala, Panfilo S. Belo*, Elveda Smith,
and Miriam Saltmarch

Department of Nutrition and Food Science

San Jose State University

1 Washington Square

San Jose, CA 95192

* Address questions and comments to: Dr. Panfilo Belo,
Department of Nutrition and Food Science, San Jose State
University, 1 Washington Square, San Jose, CA 95192,
(408)924-3108.

ABSTRACT

Ground dehulled green gram flakes were subjected to batchwise hexane extraction, and the resulting defatted meal was used to prepare flour, concentrate, and isolate. The protein content, on a dry basis, of the defatted flour, concentrate, and isolate were 25.3, 73.0, and 92.2 %, respectively. The percent protein solubility of the three products was maximum at pH 9.0 and minimum at pH 4.5. The flour showed the highest percent protein solubility (82.5 at pH 9.0 and 9.5 at pH 4.5), and the isolate showed the lowest (62.8 at pH 9.0 and 4.8 at pH 4.5). The emulsifying and foaming properties of the products were higher at pH 9.0 and lower at pH 4.5. At both pH conditions, the isolate exhibited the highest emulsifying capacity (by absorbing 52.3 and 11.8 ml oil/g of sample at pH 9.0 and pH 4.5, respectively) followed by the concentrate and flour. There was no significant difference in the percent volume increase of foam produced by the flour, concentrate, and isolate. However, the isolate showed the lowest percent drip (43.0 and 92.9 ml leakage/100 g of foam at pH 9.0 and 4.5, respectively). A significant difference was found between the in-vitro protein digestibility values for the isolate, concentrate, and flour which were 93.6, 86.2, and 77.1 %, respectively.

INTRODUCTION

The green gram (Vigna radiata [L] Wilczek) has shown potential as an important source of protein in Southeast Asia, Central Africa, the warmer parts of China, and the United States. The plant grows on all types of soils and under varying climatic conditions. It is a short duration crop and has wide adaptability. The yields are 600 kg/hectare (Adsule, Kadam, & Salunkhe, 1986). Green gram has 22.5%, by dry weight, protein and is rich in lysine. It costs about less than one dollar per pound; therefore, it is a cheap source of protein and can be used in the preparation of cheap and nutritious baby foods (Mizrahi et al., 1976). Among the commonly cultivated pulses, i.e., chick pea, cowpea, and mung bean, proteins in green gram have a more balanced amino acid composition (Deshmukh & Sohonie, 1965).

Although the potential of green gram as a protein-rich source has stimulated research interest in recent years, the database for this legume needs to be expanded to facilitate efficient utilization of its protein products. Since green gram seeds may be widely used as a protein source, it is of interest to understand fully the functional properties of its protein constituents. Based on the development of soy protein, ingredient proteins should have acceptable intrinsic properties such as flavor,

texture, color, good nutritional value, and the requisite functional properties for a variety of intended applications (Hermannson, 1979; Kinsella, 1979). Properties such as solubility, viscosity, texture, emulsion, foam, and gel characteristics are very essential in the technology and applications of green gram proteins.

Knowledge of these properties is important to facilitate the efficient utilization of this high-protein food source. This study was undertaken to determine the solubility, emulsification, foaming properties, and in-vitro digestibility of green gram protein products.

MATERIALS AND METHODS

Sample Preparation. Five pounds (2.25 kg) of dehulled green gram were purchased from a local commercial dealer. Defatted dehulled green gram flour was prepared by grinding dehulled seeds in a Straub model 4E grinding mill and extracting the flour 3 times with hexane at room temperature. The flour was air dried and re-ground using a Scienceware micro-mill to pass a 100-mesh screen.

Protein concentrate was prepared from defatted dehulled flour, using an iso-electric precipitation method described by Wolf (1970). The aqueous dispersion of defatted flour (1:100 w/v) was adjusted to pH 9.0 with 1 M NaOH, and the resulting mixture was centrifuged for 10 minutes (IEC clinical centrifuge, maximum speed). The

extraction process was repeated, and the combined supernatants were adjusted to pH 4.5 with 1 M HCl and centrifuged for 10 minutes. The precipitate was air dried overnight at room temperature and ground.

The procedure used in the preparation of the protein isolate was that described by Wolf (1970) and is shown in Figure 1. Seventy grams of defatted flour were dispersed in 700 ml of distilled water, adjusted to pH 9.0, and stirred for 30 minutes. Insoluble polysaccharides and other high molecular weight components were removed by centrifuging the extract for 20 minutes at 12,000xg. The extract was passed through a filter, and the filtrate was saved. The precipitate was then washed with 350 ml distilled water for 30 minutes, while maintaining a pH between 8.5 and 9.0. After centrifuging for 20 minutes at 12,000xg, the supernatant was removed through a filter and combined with the previous supernatant; the precipitate was discarded. The pH of the combined supernatants was adjusted to 4.5 to precipitate the protein. The mixture was centrifuged for 20 minutes at 12,000xg, and the resulting precipitate was washed with 86% ethyl alcohol at a ratio of 25:1 (alcohol:protein isolate) for four hours. The pellets were air dried overnight at room temperature and ground to a powder with a mortar and pestle. The

resulting dried protein isolate was used in subsequent analyses.

Experimental Design. Three batches each of green gram concentrate and isolate were prepared separately from the defatted flour. The three batches were mixed together and used in subsequent analyses. Proximate analysis of the flour, concentrate, and isolate was performed in triplicate according to the official methods of the Association of Official Analytical Chemists (AOAC, 1984).

Solubility, emulsification, and foaming properties of the flour, concentrate, and isolate were investigated to observe which of the three protein products show the best functional properties. 24 samples each of the flour, concentrate, and isolate were used to investigate their solubility properties at pH conditions ranging from 2.0 to 10.0; samples were evaluated in triplicate at each pH condition. The emulsification and foaming properties of the products were studied at pH conditions where their solubility was the highest and lowest based on the solubility results. Percent in-vitro protein digestibility (IVPD) of the flour, concentrate, and isolate was measured to determine the effectiveness of the extraction and isolation procedures in the preparation of the concentrate and isolate from the defatted flour. Aqueous suspensions of the protein products (6.25 mg protein/ml of the product)

were used in triplicate in studying the IVPD of the flour, concentrate, and isolate.

Proximate Analysis. Analysis of crude protein, crude fat, ash, moisture, starch, and total carbohydrates (by the difference method) were performed on the flour, concentrate, and isolate according to official methods of the Association of Official Analytical Chemists (AOAC, 1984).

Solubility Determination. The protein solubility was determined at room temperature using 1% (w/v) aqueous protein dispersions according to the method of Morr et al. (1985). A 50 ml suspension was used for each determination. The pH of the aqueous dispersions containing 0.1 M NaCl was adjusted to 2, 4, 4.5, 6, 7, 8, 9, and 10 using dilute solutions of HCl and NaOH. Dispersions were mixed using the magnetic stirrer for one hour; after which the samples were centrifuged for 10 minutes at 12,000xg. The amount of protein in the solution was then determined using the micro Kjeldahl method (AOAC, 1984).

Emulsifying Properties. Emulsifying properties were determined at the pH at which solubility of the green gram products was highest and lowest according to Beuchat (1977). 8% (w/v) aqueous protein dispersions containing 0.1 M NaCl were adjusted to pH 4.5 and 9.0. A 50 ml

suspension of each product was blended in an Osterizer blender at high speed for 30 seconds before oil was added. Using a buret, corn oil was added at a rate of 0.5 ml per second until the emulsion breakpoint was reached. The breakpoint was defined subjectively as the point at which emulsion coalescence broke to yield liquid separation and a change in consistency (McWatters & Cherry, 1977). The type of emulsion produced was determined according to McWatters and Cherry (1977), based on actual viscosity readings of the emulsions using a Brookfield viscometer. When the viscosity of the emulsion was 160,000 centipoises (cps), the consistency was described as very thick, mayonnaise like; 112,300 cps, thick, mayonnaise like; 60,160 to 70,880 cps, semi thick, mayonnaise like; 35,680 to 47,680 cps, very thick, salad dressing like; 17,280 to 26,080 cps, semi thick, salad dressing like; and 5,760 to 12,960 cps, thin pourable suspension.

Foaming Properties. Foaming properties were determined according to the method described by Eldridge et al. (1963b) with a modification. 1% (w/v) aqueous protein isolate dispersions containing 0.1 ml NaCl were adjusted to pH 4.5 and 9.0. A 100 ml suspension was used for each determination. The dispersions were whipped in an Osterizer blender for 15 minutes at room temperature. Volumes were recorded before and after whipping, and the

percentage volume increase was calculated according to Lin and Humbert (1974). Foam samples were inverted and allowed to drip through nylon netting into pre-weighed beakers (Baldwin & Sinthavali, 1974). The weight of liquid released from foam after 30 min was determined and calculated as percentage drip based on the original weight of the foam.

In-vitro Protein Digestibility (IVPD). The multi-enzyme technique of Hsu et al. (1977) was used to estimate protein digestibility. The enzymes used were porcine pancreatic trypsin (Sigma Type IX; 18,300 units/mg protein), bovine pancreatic chymotrypsin (Sigma Type II; 51 units/mg powder), and porcine intestinal peptidase (Sigma Grade III; 115 units/g powder).

In-vitro protein digestions were performed in a 37° C water bath with constant stirring. The multi-enzyme solution (1.24 mg/ml trypsin, 3.56 mg/ml chymotrypsin, 0.45 mg/ml peptidase) was held in an ice water bath prior to use. Aqueous suspensions of protein products (25 ml, 6.25 mg protein/ml) were equilibrated in a water bath to 37° C and adjusted to pH 8.0 with 0.1 N NaOH and HCl. The cold enzyme solution (2.5 ml) was added, and the pH was recorded at 30-second intervals for 10 min. Digestibility was calculated using the regression equation of Hsu et al. (1977): $Y = 210.64 - 18.103X$, where $Y = \% \text{ digestibility}$

and X = pH of the sample suspension 10 minutes after addition of the enzyme solution.

Analysis of Data. All determinations were performed in triplicate. Mean and standard deviations were calculated for all assays. The statistical difference in functional properties between the flour, concentrate, and isolate were compared using t-tests.

RESULTS AND DISCUSSION

The average yield of concentrate and isolate obtained from the defatted green gram flour was approximately 30% and 22%, respectively. The green gram protein concentrate and isolate had a slight golden-brown color. The browning is generally observed during the isolation of plant proteins and attributed to the presence of polyphenolic compounds, specially at the alkaline pH at which the concentrate and isolate are prepared (Thompson, 1977). The concentrate and isolate had no detectable odor and flavor.

The results of proximate analysis are presented in Table 1. The dry matter content of the concentrate and isolate constitute about 73.0 and 92.2% protein, respectively. The green gram flour constitutes about 89.7% dry matter, and 25.3% is protein. The fat contents of the flour, concentrate, and isolate were very low since fat equalled less than 1.5% of the original flour on a wet basis, and the flour, concentrate, and isolate were

prepared from defatted flour. The total mineral content of the flour, concentrate, and isolate on a dry basis was 3.34, 2.90, and 2.45, respectively. The total carbohydrate was obtained by difference. The isolate has the lowest amount of total carbohydrates (5.4% on a dry basis) since most water-soluble and water-insoluble carbohydrates are removed during the isolation procedures. The total carbohydrate content of the concentrate is 24.2% and that of the flour is 70.3% on a dry basis. Of the total amount of carbohydrates in the flour, starch is 83.3%. The concentrate and isolate contain lower amounts of starch compared to the flour since most of the carbohydrate is removed during extraction procedures. Although a direct comparison with values in literature is not possible because of the difference in the variety of the legume and analytical methods used, the protein, fat, ash, moisture, carbohydrate (total), and starch values are within the range reported in the literature (Adsule, Kadam, & Salunkhe, 1986; Mesallam & Hamza, 1987).

Solubility. Figure 2 shows the effect of pH on protein solubility. In the flour, concentrate, and isolate, as the pH increases from 2.0 to 4.0 or 4.5, solubility decreases; from pH 4.0 or 4.5 to pH 9.0, solubility increases; solubility gradually decreases beyond pH 9.0. At pH 2.0, the percent protein solubility of the

flour, concentrate, and isolate ranges from 49% to 68%. When the pH of the suspensions were adjusted from pH 2.0 to pH 4.5, all the products showed the lowest percent protein solubility, ranging from 4% to 9%. Although the percent protein solubility of the product improved at pH above 4.5, the products showed low solubility ranging from approximately 20% to 31%. However, beyond pH 6.0, there was a significant increase in the solubility of the three products, the maximum solubility being at pH 9.0 and ranging from 62% to 83% among the three products. Therefore, in all products, minimum solubility occurred at pH 4.5 and maximum at pH 9.0.

A similar solubility pattern was shown for soy bean (Kinsella, 1979) and mung bean protein products (Coffman & Garcia, 1979). In general, solubility of plant proteins improves with alkali treatment (Kinsella, 1979). This property is commonly used in the extraction of protein products from plants (Kinsella, 1979). However, the brown coloration of the extract was observed above pH 8.0 which is attributed to the presence of polyphenolic compounds. This phenomenon has been observed in the preparation of protein products from other legumes (Thompson, 1977). The iso-electric point of green gram proteins is between pH 4.0 and 4.5. Similar results have been reported by

Krishnamurthy and Rama Rao (1976) and Deshmukh and Sohoni (1965).

The solubility of proteins at pH 4.5 and pH 9.0 was compared, and the results are shown in Table 2. Although the number of samples used in the experiment were low, the reproductibility of the results was good which is evident by very low standard deviations. Solubility of the isolate was significantly lower at both pH conditions than that observed for the concentrate ($p \leq 0.05$) and the flour ($p \leq 0.05$), and the concentrate showed a significantly lower protein solubility (%) than the flour ($p \leq 0.05$). The low percent protein solubility of the concentrate and the isolate compared to the flour might be due to the denaturation of the proteins during the isolation and extraction of protein products from the flour. Based on the solubility values, it is evident that the isolate was denatured to a greater degree than the protein concentrate. Denaturation of proteins lowers not only their capacity to bind water but also their degree of solubility (West, 1984). Similar observations were made by Mesallam and Hamza (1987) who found that protein solubility of green gram flour was higher than its corresponding protein isolate. Abdel-Aal (1983) also reported that the flours of chick pea, feba bean, and fenugreek had higher protein solubility than the corresponding protein isolates.

Solubility of proteins is an important factor in the isolation and extraction of high-protein products such as concentrates and isolates. Solubility is also important when considering the use of proteins as ingredients in food products, particularly in beverages. Since green gram products showed about 49% to 68% protein solubility at pH 2.0, they are prospective ingredients in acidic beverages.

Emulsification. Data on the emulsifying capacity of the flour, concentrate, and isolate are presented in Table 3. The emulsifying capacity of the proteins is higher at pH 9.0 than at pH 4.5. Similar observations with mung bean proteins are reported by Coffmann and Garcia (1979). The data indicate that adjusting the pH of the dispersion from 4.5 to 9.0 resulted in approximately a four-fold increase in emulsifying capacity of the flour, concentrate, and isolate. This increase in emulsifying capacity can be attributed to high solubility of the protein products at pH 9.0 compared to pH 4.5. This relationship between protein solubility and emulsifying capacity has been observed by McWatters et al. (1976) in soy bean proteins and by Lin and Zayas (1987) in corn germ proteins.

There is a significant difference ($p \leq 0.05$) in emulsifying capacity between the flour, concentrate, and isolate at pH 9.0 and 4.5, with the isolate having the highest. A similar observation was reported by Mesallam

and Hamza (1987) for green gram flour and isolate. This is most likely due to the difference in the protein content and solubility of the three products at different pH conditions.

It is interesting to note that the type of emulsion formed differed not only among the three protein products but also at the two pH conditions. At pH 4.5, the emulsion capacity of the flour was extremely poor, forming thin, pourable suspensions (11,000 cps); the emulsion capacity of the concentrate was better, forming a semi thick, salad dressing like emulsion (23,300 cps). The emulsion capacity of the isolate at pH 4.5 was the best; the emulsion was thick and salad dressing like in consistency (35,000 cps). When the pH of the suspensions were adjusted to pH 9.0, however, the quantity of oil emulsified as well as the type of emulsions produced were dramatically improved. At pH 9.0, the emulsion formed by the flour was semi thick and mayonnaise like in consistency (60,700 cps), the emulsion formed by the concentrate was thicker than the flour and was mayonnaise like in consistency (112,300 cps), and the emulsion consistency of the isolate was much thicker (>160,000 cps) forming very thick, mayonnaise like emulsion. The differences in the type of emulsion formed among the three products and at the two pH conditions are most likely due to a difference in the amount of protein

available to bind the fat as well as to the effect of pH on protein solubility.

Foaming. Foaming capacity and foam stability of the flour, concentrate, and isolate are shown in Table 4. The percent volume increase and the percent drip of the protein foams at the two pH conditions were significantly different ($p \leq 0.05$). The data show that all the three protein products yielded greater percentage volume increase and more stable foams (lower % drip) at pH 9.0 than at pH 4.5. This difference in foaming properties is attributed to the difference in the solubility of the three protein products at both pH conditions. Similar observations were made for soy bean protein products by Eldridge et al.(1963a) and for mung bean protein isolate by Coffmann and Garcia (1979).

There is a significant difference ($p \leq 0.05$) in the stability (% drip) but no significant difference ($p \leq 0.05$) in percent volume increase of the foams produced by the three protein products at both pH conditions. The foam produced by the isolate showed the highest stability (lowest percent drip) at both pH conditions, and the foam produced by the flour showed the lowest stability (highest percent drip) at both pH conditions. The difference in stability of the three foams might be due to the difference in the protein concentration of the three products at both pH conditions, the isolate having the highest protein

content and the flour having the lowest. Eldridge et al. (1963a) observed that a 10% soy bean protein suspension produced more stable foam than a 3% soy bean protein suspension, but the percent volume increase of the two foams was not significantly different. Foam produced by green gram proteins was light beige in color and more closely resembled whipped cream than egg white.

IVPD. Data in Table 4 show the results of in-vitro digestibility. The IVPD of the flour is 77.1%, which compares favorably to some plant protein sources such as raw beans and corn-milo grain (Hsu et al., 1977). The IVPD of the concentrate is 86.2% which is similar to that of soy concentrate, and the IVPD of the isolate is 93.6% which compares favorably to soy isolate (Hsu et al., 1977). A similar IVPD was observed for green gram protein isolate by Mesallam and Hamza (1987) when pepsin followed by pancreatin digestion was used. The difference in percent IVPD between the three protein products may be due to the difference in the degree of removal of anti-nutritional factors from the flour, concentrate, and isolate during extraction procedures. Since the isolate goes through more complex procedures of processing during preparation from the defatted flour, it is more refined than the concentrate and the flour and has a higher percent of IVPD. The high IVPD of the isolate and concentrate compared to that of the

flour also suggests that the proteins of the concentrate and isolate were opened up and denatured during the isolation and extraction from the flour. As a result, the soluble denatured proteins of the isolate and concentrate might have better emulsifying and foaming capacities than the soluble compact proteins of the flour. It is theorized that this might be the reason for the high emulsifying and foaming properties of the isolate and concentrate, despite their low solubility properties. According to Hsu et al. (1977), processing can improve the digestibility of proteins by destroying anti-nutritional factors such as protease inhibitors and by changing the protein structure by denaturation.

CONCLUSIONS

The flour, concentrate, and isolate prepared from defatted dehulled green gram flour were high in protein and very low in fat content. The concentrate and isolate obtained from the defatted flour were golden-brown in color with no detectable flavor and odor. The solubility of the proteins was affected by the pH of the dispersion medium. Maximum solubility was observed at pH 9.0 and minimum solubility at pH 4.5 which is the phenomena exhibited by most legume proteins (Kinsella, 1979). Low solubility of only 4-12% between pH 4-4.5 limited the usefulness of the flour, concentrate, and isolate in foods at these pH

levels. There was a significant difference in solubility between the three proteins at pH 9.0 and pH 4.5, the isolate having the least solubility at both pH conditions, followed by the concentrate and the flour. The low solubility of the isolate followed by the concentrate compared to the flour might be due to partial denaturation of the proteins of the concentrate and isolate during extraction procedures. The isolate proteins might have undergone a higher degree of denaturation compared to the concentrate.

Emulsifying and foaming properties of the proteins were directly related to the solubility. The more soluble the flour, concentrate, and isolate, the greater both the emulsifying capacity and the stability of the foams. The isolate showed the highest emulsifying capacity and foam stability followed by the concentrate and the flour. It is theorized that the differences in emulsifying capacity and foam stability between the three proteins were due to the differences in protein content. Although not equal to whipped egg white, the isolate demonstrated good whipping ability. A creamy, relatively stable foam of pale beige color was formed from a solution of 8% protein isolate. The isolate showed the highest % IVPD (93.6%) followed by the concentrate (86.2%) and the flour (77.1%). Processing improved the digestibility of the isolate and concentrate

by destroying anti-nutritional factors such as protease inhibitors and opening the protein structure through denaturation.

Figure 1. Preparation of green gram protein isolate from defatted flour.

Figure 2. Effect of pH on solubility of 1% (w/v) aqueous dispersions of green gram protein products containing 0.1 M NaCl at room temperature (26°C).

Table 1. Proximate Analysis of the Flour, Concentrate, and Isolate from Defatted, Dehulled Green Gram on Wet Basis

constituent	flour	concentrate	isolate
	(a)	(a)	(a)
crude protein (%)	22.7 $\pm$ 0.1	67.2 $\pm$ 0.4	87.1 $\pm$ 1.3
crude fat (%)	1.0 $\pm$ 0.2	0.2 $\pm$ 0.3	0.1 $\pm$ 0.2
ash (%)	3.0 $\pm$ 0.1	2.6 $\pm$ 0.01	2.2 $\pm$ 0.2
moisture (%)	10.3 $\pm$ 0.2	7.9 $\pm$ 0.3	5.6 $\pm$ 0.2
carbohydrates			
(total) (%)	63.0	22.1	5.0
starch (%)	52.6 $\pm$ 0.3	17.3 $\pm$ 0.7	3.8 $\pm$ 2.5

(a) mean $\pm$ standard deviation of three determinations.

Table 2. Comparison of Solubility in 1% (w/v) Aqueous Dispersions of Green Gram Flour, Concentrate, and Isolate Containing 0.1 M NaCl at Room Temperature (26° C)

	pH	flour	concentrate	isolate
		(a)	(a)	(a)
solubility	4.5	9.5 ± 0.8	6.2 ± 1.5	4.8 ± 1.0
(% protein dissolved)	9.0	82.5 ± 2.3	70.0 ± 1.5	62.8 ± 3.0

(a) value is the average of triplicate samples ± standard deviation.

Table 3. Effect of pH on Emulsifying Property in 8% (w/v) Aqueous Dispersions of Green Gram Protein Products at Room Temperature (26° C) and 0.1 M NaCl

sample	pH of dispersion system	emulsifying (a) capacity (ml oil/gm sample)	type of emulsion
flour	4.5	6.8 $\pm$ 0.3	thin pourable suspension.
	9.0	24.4. $\pm$ 0.5	semi-thick mayonnaise like
concentrate	4.5	9.0 $\pm$ 0.5	semi-thick salad dressing like
	9.0	38.2 $\pm$ 0.3	thick mayonnaise like
isolate	4.5	11.8 $\pm$ 0.7	thick salad dressing like
	9.0	52.3 $\pm$ 1.4	very thick mayonnaise like

(a) mean $\pm$ standard deviation of three determinations.

Table 4. Effect of pH on Foaming Properties in 1% (w/v)
Aqueous Dispersions of Green Gram Protein Products at Room
Temperature (26° C) and 0.1 M NaCl

sample	pH of suspension	% volume increase (a)	% drip (ml leaked /100 gm foam) (a)
flour	9.0	80.0 ± 2.0	62.8 ± 5.3
	4.5	46.0 ± 4.0	92.9 ± 3.5
concentrate	9.0	85.0 ± 4.6	58.7 ± 4.2
	4.5	56.0 ± 4.0	88.0 ± 3.0
isolate	9.0	92.3 ± 6.4	43.0 ± 3.0
	4.5	58.6 ± 6.1	81.0 ± 2.6

(a) mean ± standard deviation of three determinations.

Table 5. In-vitro Protein Digestibility (IVPD) of Green Gram Protein Products

sample	% IVPD
flour	77.1 $\pm$ 0.3
concentrate	86.2 $\pm$ 0.2
isolate	93.6 $\pm$ 0.3

(a) Mean $\pm$ standard deviation of three determinations.

LITERATURE CITED

- Abdel-Aal, E.M. Chemical and technological studies on some legumes and rice as a source of proteins; M.S. Thesis, University of Alexandria, 1983.
- Adsule, R.N.; Kadam, S.S; Salunkhe, D.K. Chemistry and technology of green gram (Vigna radiata [L] Wilczek). CRC Cri. Rev. in Food Sci. Nutr. 1986, 25, 73-105.
- AOAC. Official Methods of Analysis, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984.
- Baldwin, R.E.; Sinthavalai, S.J. Functional evaluation of proteins in food systems. Food Sci. 1974, 39, 880-889.
- Beuchat, L.R. Functional and electrophoretic characteristics of succinulated peanut flour proteins. J. Agric. Food Chem. 1977, 25, 258-260.
- Coffmann, C.W.; Garcia, V.V. Functional properties and amino acid content of a protein isolate from mung bean flour. J. Food Tech. 1979, 12, 473-484.
- Deshmukh, A.D.; Sohonie, K. Proteins of green gram. J. Nutr. Dietetic 1965, 2, 183-186.
- Eldridge, A.C.; Hall, P.K.; Wolf, W.J. Stable foams from unhydrolyzed soybean protein. J. Food Tech. 1963a, 17, 1592-1598.
- Eldridge, A.C.; Wolf, W.J.; Nash, A.M.; Smith, A.K. Alcohol washing of soybean protein. J. Agric. Food Chem. 1963b, 11, 323-329.

- Hermansson, A.M. Methods of studying functional characteristics of vegetable proteins. J. Am. Oil Chem. Soci. 1979, 56, 242-246.
- Hsu, H.W.; Vavak, D.L.; Satterlee, L.D.; Miller, G.A. A multienzyme technique for estimating protein digestibility. J. Food Sci. 1977, 42, 1269-1273.
- Kinsella, J.E. Functional properties of soy beans. J. Am. Oil Chem. Soci. 1979, 56, 242-247.
- Krishnamurthy, K.S.; Rama Rao, R.G. Studies on the proteins of green gram. Ind. Food Packer 1976, 10, 28-33.
- Lin, M.J.Y.; Humbert, E.S. Certain functional properties of sunflower meal product. J. Food Sci. 1974, 39, 368-371.
- Lin, C.S.; Zayas, J. Protein solubility, emulsifying stability and capacity of two defatted corn germ proteins. J. Food Sci. 1987, 52, 1615-1624.
- McWatters, K.H.; Cherry, J.P. Emulsification, foaming and protein solubility properties of defatted soybean, peanut, field pea, and pecan flours. J. Food Sci. 1977, 42, 1445-1448.
- McWatters, K.H.; Cherry, J.P.; Holmes, M.R. Influence of suspension medium, and pH on functional and protein properties of defatted peanut meal. J. Agric. Food Chem. 1976, 24, 517-519.

- Mesallam, A.S; Hamza, M.A. Studies on green gram protein concentrate and flour. Pl. Foods Hum. Nutr. 1987, 37, 17-27.
- Mizrahi, S.; Zimmerman, G.; Berk, Z.; Cogan, U. The use of isolated proteins in bread. Cereal Chem. 1976, 44, 193-198.
- Morr, C.V.; German, B.; Kinsella, J.E.; Regenstein, J.M.; Van Buren, J.P.; Kilara, A.; Lewis, B.A.; Mangino, M.E. A collaborative study to develop a standardized food protein solubility procedure. J. Food Sci. 1985, 50, 1715-1718.
- Thompson, L.U. Preparation and evaluation of mung bean protein isolates. J. Food Sci. 1977, 42, 202-206.
- West, S.I. Food proteins: sources and properties. J. Chem. Tech. Biotech. 1984, 34, 176-181.
- Wolf, W.J. Soybean proteins: Their functional, chemical, and physical properties. J. Agric. Food Chem. 1970, 18, 969-976.
- FUNDING NOTE: Funds for this research were provided through a Mini-Research Grant from The San Jose State University, School of Applied Arts and Sciences.

CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Green gram concentrate and isolate were prepared from defatted dehulled green gram flour. The flour, concentrate, and isolate were evaluated for proximate composition, solubility, emulsification, and foaming properties and for in-vitro digestibility. The values were compared between the three products. The average yield of concentrate and isolate obtained from the flour was approximately 30% and 22%, respectively, and had a slight golden-brown color. The isolate was higher in protein, about one and a quarter times (92.2%) in the concentrate (73.0%) and approximately four times that of the flour (25.3%). The fat content of the three products was low since the original flour had only 1.5% fat on a dry basis and because the protein products were prepared from defatted flour. The total carbohydrate (obtained by the difference method), starch, and mineral (% ash) contents of the flour were the highest followed by the concentrate and the isolate.

The solubility of the flour, concentrate, and isolate was affected by pH of the dispersion medium. As the pH of the medium increased from 2.0 to 4.0 or 4.5, the solubility of the three products decreased; from pH 4.0 or 4.5 to pH

9.0, the solubility increased after which there was a gradual decrease in solubility beyond pH 9.0. Therefore, the highest solubility occurred at pH 9.0 and the lowest at pH 4.5 (the isoelectric point of most oil seeds) (Kinsella, 1979). Although the solubility properties of flour, concentrate, and isolate showed a similar pattern as affected by pH, there is a significant difference in the amount (%) of protein, soluble at the different pH conditions, between the three products. The isolate exhibited the lowest percent protein solubility both at pH 4.5 and at pH 9.0, followed by the concentrate and flour. This might be due to some degree of denaturation of the green gram proteins during the isolation and extraction of the concentrate and isolate from the flour, the isolate being denatured to a greater degree than the concentrate.

The flour, concentrate, and isolate had a four-fold increase in emulsifying capacities, expressed as ml oil/g sample when the pH of the dispersion was adjusted from 4.5 to 9.0. Similar observations have been reported for soy bean by McWatters et al. (1976) and mung bean proteins by Coffmann and Garcia (1977). There was a significant difference in emulsifying capacity between the three products of green gram. At pH 4.5 and 9.0, the emulsifying capacity of the isolate was the highest, and that of the flour was the lowest. Mesallam and Hamza (1987) observed a

similar relationship between the emulsifying capacity of green gram flour and its isolate. The type of emulsion formed also differed among the three products and at pH 4.5 and 9.0. These ranged from a thin, pourable suspension at pH 4.5 for the flour to very thick and mayonnaise like system at pH 9.0 for the isolate.

The three protein products yielded greater percentage volume increase and more stable foams (lower % drip) at alkaline pH (9.0) at which the solubility of the products were the highest, than at the isoelectric pH (4.5) at which the solubility is the lowest. There was no significant difference in % volume increase of the foams produced by the flour, concentrate, and isolate at either pH. However, at pH 4.5 and 9.0, the foam produced by the isolate showed the highest stability, and the foam produced by the flour showed the lowest. Foam produced by the three products was light beige in color and more closely resembled whipped cream than egg white.

There was a significant difference in the in-vitro protein digestibility of the flour, concentrate, and isolate. The isolate had the highest percent IVPD (93.6%) followed by the concentrate (86.2%) and flour (79.1%). Processing can improve the digestibility of proteins by destroying anti-nutritional factors such as protease

inhibitors and opening of the protein structure through denaturation (Hsu et al., 1977).

Recommendations

Further research on the methods used in the preparation of green gram concentrate and isolate is recommended, since there was some degree of denaturation of concentrate and isolate proteins during the isolation and extraction procedures. This was made evident by the low percent solubility of the isolate and the concentrate compared to the flour, in the solubility studies. Further research is also recommended to determine the cause of the golden-brown coloration of the concentrate and isolate prepared from the defatted flour. Studies on the effect of factors, not analyzed in this study, on the solubility of green gram proteins would make an important contribution to the knowledge of solubility properties of legume proteins. Of the two pH conditions (pH 4.5 and pH 9.0) at which the emulsifying and foaming properties of green gram flour, concentrate, and isolate were studied, pH 9.0 is not a practical pH in most food systems. To use green gram products as emulsifying and foaming agents in food systems, further research is necessary to study the emulsifying and foaming properties of the flour, concentrate, and isolate at conditions that are suitable to most food systems, such as pH, temperature, and ionic strength. A better

evaluation of the effectiveness of the extraction and isolation procedures used in the preparation of the concentrate and the isolate from the flour would have been obtained if the emulsification and foaming properties of the isolate and concentrate (although not possible for the flour) were studied on an equal protein concentration basis.

To accelerate the utilization of green gram protein products, further research is recommended to determine the other functional properties (e.g., water absorption, viscosity, gelation, cohesion-adhesion, elasticity, fat adsorption, flavor-binding, and color control) of the flour, concentrate, and isolate.

Although the in-vitro digestibility of green gram protein products is comparable to other plant protein sources currently in use, the in-vitro results need to be confirmed with animal feeding studies. Further research is also necessary to determine the effects of factors such as the degree of processing and the presence of anti-nutritional factors on the digestibility of green gram protein products. Practical testing in complicated food systems needs to be done to establish the role of green gram flour, concentrate, and isolate as nutritional and functional components in foods.

REFERENCES

- Abdel-Aal, E.M. (1983). Chemical and technological studies on some legumes and rice as a source of protein.
Unpublished master's thesis, University of Alexandria, Alexandria, Egypt.
- Adsule, R.N., Kadam, S.S., & Salunkhe, D.K. (1986).
Chemistry and technology of green gram (Vigna radiata [L] Wilczek). CRC Critical Reviews in Food Science and Nutrition, 25, 73-105.
- Akeson, W.R., & Stahmann, M.A. (1964). A pepsin pancreatin digest index of protein quality evaluation. Journal of Nutrition, 83, 257-262.
- Aoki, H., Teneyama, O., & Inami, M. (1980). Emulsifying properties of soy protein: Characteristics of 7S and 11S proteins. Journal of Food Science, 45, 534-538.
- Association of Official Analytical Chemists. (1984).
Official method of analysis (13th ed.). Washington, DC: AOAC.
- Baldwin, R.E., & Sinthavali, S. (1974). Functional evaluation of proteins in systems. Journal of Food Science, 39, 880-889.
- Beuchat, L.R. (1977). Functional and electrophoretic characteristics of succinylated peanut flour protein. Journal of Agriculture and Food Chemistry, 25, 258-260.

- Chatterjee, S.R., & Abrol, Y.P. (1975). Amino acid composition of new varieties of cereals and pulses and nutritional potential of cereal-pulse combinations. Journal of Food Science and Technology, 12, 221-224.
- Cherry, J., McWatters, K., & Beuchat, L. (1979). Oil seed protein properties related to functionality in emulsions and foams. In A. Pour-El (Ed.), Functionality and protein structure (pp. 158-162). Washington, DC: American Chemical Society.
- Coffmann, C.W., & Garcia, V.V. (1979). Functional properties and amino acid content of a protein isolate from mung bean flour. Journal of Food Technology, 12, 473-484.
- De Kanterewicz, R.J., Elizalda, B.E., Pilosof, A.M.R., & Bartholomai, G.B. (1987). Water-oil absorption index (WOAI): A simple method for predicting the emulsifying capacity of food proteins. Journal of Food Science, 52, 1381-1383.
- Derbyshire, E., Wright, D.J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. Phytochemistry, 15, 3-8.
- Deshmukh, A.O., & Sohoni, K. (1965). Proteins of green gram. Journal of Nutrition and Dietetics, 2, 183-186.
- Eldridge, A.C., Hall, P.K., & Wolf, W.J. (1963a). Stable foams from unhydrolyzed soybean protein. Journal of

- Food Technology, 17, 1592-1598.
- Eldridge, A.C., Wolf, W.J., Nash, A.M., & Smith, A.K.
(1963b). Alcohol washing of soybean protein. Journal of Agriculture and Food Chemistry, 11, 323-329.
- Hanumantha, R.K., & Subramanian, N. (1970). Essential amino acid composition of commonly used Indian pulses by paper chromatography. Journal of Food Science and Technology, 7, 31-34.
- Hermansson, A.M. (1979). Methods of studying functional characteristics of vegetable proteins. Journal of American Oil Chemists' Society, 56, 242-246.
- Hsu, H.W., Vavak, D.L., Satterlee, L.D., & Miller, G.A.
(1977). A multi-enzyme technique for estimating protein digestibility. Journal of Food Science, 42, 1269-1273.
- Huang, Y.T., & Kinsella, J.E. (1987). Effects of phosphorylation on emulsifying and foaming properties and digestibility of yeast protein. Journal of Food Science, 52, 6-10.
- Hutton, C.W., & Campbell, A.M. (1977). Functional properties of soy concentrates and soy isolates in simple systems and in a food system. Journal of Food Science, 42, 457-460.
- Inkelaar, P., & Fortuin, J. (1969). Determining the emulsifying and emulsion stabilizing capacity of protein and meat additives. Food Technology, 23, 103-105.

- Johnson, D.W. (1970). Functional properties of oil seed proteins. Journal of American Oil Chemists' Society, 47, 402-405.
- Kinsella, J.E. (1979). Functional properties of soy beans. Journal of American Oil Chemists' Society, 56, 242-247.
- Kitabatake, N., & Doi, E. (1988). Surface tension and foamability of protein and surfactant solutions. Journal of Food Science, 53, 1542-1545.
- Kramer, A., & Kwee, W.H. (1977). Functional and nutritional properties of tomato protein concentrates. Journal of Food Science, 42, 207-209.
- Krishnamurthy, K.S., & Rama Rao, R.G. (1976). Studies on the proteins of green gram. Indian Food Packer, 10, 28-33.
- Lawhon, J.T., & Cater, C.M. (1971). Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cottonseed. Journal of Food Science, 36, 372-378.
- Lin, M.J.Y., & Humbert, E.S. (1974). Certain functional properties of sunflower meal product. Journal of Food Science, 39, 368-371.
- Lin, C.S., & Zayas, J. (1987). Protein solubility, emulsifying stability and capacity of two defatted corn germ proteins. Journal of Food Science, 52, 1615-1624.
- Mattil, K.F. (1971). The functional requirements of protein

- for foods. Journal of American Oil Chemists' Society, 48, 447-449.
- McWatters, K.H., & Cherry, J.P. (1977). Emulsification, foaming, and protein solubility properties of defatted soybean, peanut, field pea, and pecan flours. Journal of Food Science, 42, 1445-1448.
- McWatters, K.H., Cherry, J.P., & Holmes, M.R. (1976). Influence of suspension medium and pH on functional and protein properties of defatted peanut meal. Journal of Agricultural and Food Chemistry, 24, 517-520.
- Mesallam, A.S., & Hamza, M.A. (1987). Studies on green gram protein concentrate and flour. Plant Foods for Human Nutrition, 37, 17-27.
- Mizrahi, S., Zimmerman, G., Berk, Z., & Cogan, U. (1976). The use of isolated proteins in bread. Cereal Chemistry, 44, 193-198.
- Morr, C.V., German, B., Kinsella, J.E., Regenstein, J.M., Van Buren, J.P., Kilara, A., Lewis, B.A., & Mangino, M.E. (1985). A collaborative study to develop a standardized food protein solubility procedure. Journal of Food Science, 50, 1715-1718.
- Rakosky, J. (1987). Soy grits, flour, concentrates, and isolates in meat products. Journal of American Oil Chemists' Society, 51, 123-126.
- Salunkhe, D.K. (1982). Legumes in human nutrition: Current

- status and future research needs. Current Science, 51, 387-390.
- Sathe, S.K., & Salunkhe, D.K. (1981). Functional properties of the great northern bean (Phasedus vulgaris L.) proteins: Emulsion, foaming, viscosity, and gelation properties. Journal of Food Science, 46, 71-73.
- Saunders, R.M., Connor, M.A., Booth, A.N., Bickoff, E.M., & Kohler, G.O. (1973). Measurement of digestibility of alfalfa protein concentration by in-vivo and in-vitro methods. Journal of Nutrition, 103, 530-536.
- Sosulski, R., Humbert, E.S., Bui, K., & Jones, J.D. (1976). Functional properties of rapeseed flours, concentrate and isolate. Journal of Food Science, 41, 1349-1352.
- Thompson, L.U. (1977). Preparation and evaluation of mung bean protein isolates. Journal of Food Science, 42, 202-206.
- West, S.I. (1984). Food proteins: Sources and properties. Journal of Chemical Technology and Biotechnology, 34, 176-181.
- Wolf, W.J. (1970). Soybean proteins: Their functional, chemical, and physical properties. Journal of Agriculture and Food Chemistry, 18, 969-976.