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Functional properties of pigeonpea protein isolate

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Functional properties of pigeonpea protein isolate

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San Jose State University, 1991

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FUNCTIONAL PROPERTIES OF PIGEONPEA PROTEIN ISOLATE

A Thesis

Presented to

The Faculty of the Department of
Nutrition and Food Science
San Jose State University

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

By

Madhavi Galivanche

May, 1991

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ABSTRACT

FUNCTIONAL PROPERTIES OF PIGEONPEA PROTEIN ISOLATE

by Madhavi Galivanche

The protein isolate was prepared from dehusked split pigeonpeas. The protein solubility profiles of the flour and the isolate were studied at pH range 2.0-12.0. The other functional properties studied were water absorption capacity, fat absorption capacity, emulsion, and foaming properties of the isolate.

Pigeonpea protein isolate was rich in protein equalling 88.0% on the dry weight basis. The yield of protein isolate and the recovery of protein from the full fat flour were approximately 20% and 76%, respectively. The isoelectric point of the protein isolate was at pH 4.6. The water absorption capacity was 6.5-7.0 gm H₂O/gm isolate at acid pH and 2.2-4.0 gm H₂O/gm isolate at neutral pH. The fat absorption capacity was 3.2 gm oil/gm isolate. The emulsion and foaming capacities were 118.0 gm oil/gm isolate and 37.39% by volume, respectively, at the neutral pH.

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PREFACE

This thesis is written in a publication style. The second chapter is written in journal style format in accordance with the January 1991 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, 1989.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Pigeonpea (Cajanus cajan) is an important legume in semi arid tropical and subtropical countries of the world (Singh et al., 1984a; Yadav, 1983). It is one of the major legumes which contributes significantly to the food protein supply in the countries where it is cultivated. Its economic importance in regions of the world such as India, East Africa, the Caribbean, and Latin America, is now well recognized.

Protein content of pigeonpea is 15.0-28.0% (Salunkhe, 1986) and it is rich in lysine (Oshodi & Ekperigin, 1989). However, it is not being utilized as a source of high protein products, such as concentrates and isolates even in the areas where it is grown abundantly, due to lack of technology. While the composition and the nutritional value of pigeonpea have been the subject of numerous studies (Mahadevappa & Raina, 1978; Modi & Kulkarni, 1976; Oshodi & Ekperigin, 1989; Rao & Subramanian, 1970; Salunkhe, 1986; Singh et al., 1984a, 1990), the literature on the functionality of the proteins is limited. Since the use of protein concentrate and isolate products is now recognized as an effective way of utilizing the legume

proteins (Salunkhe, 1986), there is a need to expand the data on functional properties.

Functional properties are the primary factors in determining the utility of proteins as ingredients in various food products (Crenwelge et al., 1974). Systematic determination of functional properties is essential in predicting the way in which a new protein product will behave in specific food systems (Kinsella, 1976). The ultimate success of utilizing plant protein as an ingredient depends largely upon the beneficial qualities which it imparts to the food systems (McWatters & Cherry, 1977). This research study was designed to determine some of the functional properties of pigeonpea protein isolate such as solubility, water absorption capacity, fat absorption capacity, and emulsion and foaming properties at two pH conditions which are most commonly used in food systems i.e., pH 4.0-5.0 and pH 6.5-7.0. These properties are important in food preparations like beverages, bakery products, salad dressings, and whipped toppings.

Objectives

The primary objective of the study was to determine the functional properties of protein isolate prepared from matured, dehusked split pigeonpeas. The study was conducted in two phases. The objective of phase I was to

establish the optimum conditions for the maximum recovery of protein from pigeonpea flour. The specific experiments performed in phase I include 1) proximate analysis of the full fat flour, 2) calibration of Lowry method of protein estimation against Kjeldahl, and 3) determination of protein solubility behavior of pigeonpea flour. The experiments performed in Phase II of the study include 1) preparation of pigeonpea protein isolate, 2) proximate analysis of the protein isolate, and 3) determination of the functional properties of the protein isolate. The functional properties studied were protein solubility, water absorption capacity, fat absorption capacity, and emulsion and foaming properties at two pH conditions (acidic, i.e., pH 4.0-5.0 and neutral, i.e., pH 6.5-7.0).

Significance of the Study

Protein malnutrition is a major nutritional problem in the underdeveloped countries. The problem of providing protein to the expanding world population is second only to the world hunger problem. With an increase in population, there is an increase in demand for the protein-rich foods (Ige et al., 1984). According to Salunkhe (1986), this demand can be met by utilizing the protein from plant sources.

Pigeonpea is one such source which is relatively high

in protein. The mature and green seeds of pigeonpeas are consumed in the countries where they are grown. However, the protein products are not being utilized as ingredients in food systems because data are limited on the functional properties (Salunkhe, 1986). It is theorized that studies on the functional properties of pigeonpea protein products will increase the use of these protein products in the food systems.

Review of Literature

The worldwide acreage of cultivation and production of pigeonpeas in 1975 (Salunkhe, 1986) are shown in Table 1. Approximately 85-90% of the total world production occurs in India (Singh et al., 1990). The mature pigeonpea seeds are hard and smooth, round to oval in shape with a raised hilum, and reddish yellow in color. The seed is composed of an outer layer (testa or seed coat), the two cotyledons, and the germ which constitute about 10.0-18.0%, 85.0-89.9%, and 0.7-1.5% of the total seed weight, respectively (Salunkhe, 1986). The protein, fat, carbohydrate, vitamins, and minerals are concentrated in the cotyledons.

Nutrient Composition

Proteins and Amino Acids

The protein content of mature pigeonpea seeds ranges from 15.0 to 28.0% (Oshodi & Ekperigin 1989; Salunkhe,

Table 1

Acreage of Cultivation, Yield/Hectare, and Production of
Pigeonpea in the World in 1975

Country	Area		
	cultivated	Yield	Production
	$\times 10^3$ ha	Kg/ha	$\times 10^6$ Kg
World	2803	699	1960
India	2540	716	1818
Uganda	99	406	40
Dominican Republic	13	2194	29
Burma	68	351	24
Malawi	35	570	20
Tanzania	20	500	10
Puerto Rico	6	742	4
Hawaii	7	538	4
Venezuela	9	449	4
Trinidad	2	1667	3

Note. From "Pigeonpea as an Important Food Source" by D. K. Salunkhe, 1986, CRC Critical Reviews in Food Science and Nutrition, 23, p. 105.

1986; Singh & Jambunathan, 1981; Taha, 1987).

Approximately 90% of the total proteins is in the cotyledons (Salunkhe, 1986). Although the germ is rich in proteins, its contribution to the total protein is negligible, since the germ constitutes only 0.7-1.5% of the total seed weight (Salunkhe, 1986). The genetic makeup, the environmental factors, the degree of maturity, and the agricultural practices influence the protein content in pigeonpeas (Salunkhe, 1986).

A protein fractionation study (Table 2) (Singh et al., 1990) showed that the globulins constitute the major portion of total proteins (60-66%) and are mostly concentrated in the cotyledons and the germ (Salunkhe, 1986). Glutelins, which constitute about 19.0-23.0% of the total protein (Singh et al., 1981, 1990), are primarily present in the seed coat (Singh et al., 1981). The albumin fraction is 8.0-9.1% (Singh et al., 1990), and the prolamin fraction is 2.1-3.6% (Singh et al., 1981, 1990).

The amino acid composition of four different cultivars of pigeonpea proteins is shown in Table 3 (Singh et al., 1984a). The data show that pigeonpea proteins are rich in lysine, phenylalanine, and arginine, but deficient in methionine, cystine, and tryptophan. Similar results were reported by Oshodi and Ekperigin (1989), Salunkhe (1986),

Table 2

Protein Fractions (gm/100gm Protein) of Various Pigeonpea Genotypes

Genotype	Albumin	Globulin	Glutelin	Prolamin
HPL 8	9.1	63.5	20.2	2.9
HPL 40	8.0	66.2	19.7	3.2
C 11	7.7	60.5	23.3	3.6
ICPL 211	8.6	60.3	22.8	2.1

Note. From "Nutritional Quality Evaluation of Newly Developed High Protein Genotypes of Pigeonpea (Cajanus cajan)" by U. Singh, R. Jambunathan, K. Saxena, and N. Subramanyan, 1990, Journal of Science Food and Agriculture, 50, p. 205.

Table 3

Amino Acid Composition (gm/100gm Protein) of Four Cultivars of Mature Pigeonpea Seeds

Amino Acid	ICPL-128	ICPL-6997	ICP-7035	C-11
Lysine	6.90	6.25	6.73	6.96
Arginine	7.24	5.58	5.66	6.24
Aspartic Acid	10.34	9.13	8.91	9.16
Threonine	3.59	3.52	3.65	3.21
Glutamic Acid	18.64	17.23	18.71	18.46
Alanine	3.61	3.37	3.85	4.23
Cystine	0.90	0.80	1.19	0.11
Valine	4.03	3.07	3.78	3.82
Methionine	1.30	1.40	1.39	1.18
Isoleucine	3.23	3.13	3.14	3.34
Leucine	7.02	6.81	6.35	6.82
Tyrosine	3.03	2.60	2.94	2.64
Phenylalanine	9.22	9.19	9.54	8.18
Tryptophan	0.86	0.73	0.67	0.80

Note. From "Nutritional Quality of Vegetable Pigeonpeas (Cajanus cajan): Dry Matter Accumulation, Carbohydrates and Proteins" by U. Singh, K. C. Jain, R. Jambunathan, and D. G. Faris, 1984a, Journal of Food Science, 49, p. 802.

Singh et al. (1981, 1984a, 1990), and Taha (1987). The amino acid composition of the germ proteins is nutritionally better than that of the cotyledons (Salunkhe, 1986). The essential amino acid content of germ protein is 39.1 g/16g nitrogen (Rao & Subramanian, 1970).

Carbohydrates

The total carbohydrate content of pigeonpeas is about 57.3-58.7% (Salunkhe, 1986). The starch content of mature pigeonpeas is 50.8-55.2% of the total carbohydrates (Singh et al., 1984a), and amylopectin and amylose contents are 26.0-27.0% and 70.0-73.0% of total starch, respectively (Modi & Kulkarni, 1976). According to Singh et al. (1989), the starch is suitable for noodle preparation.

Pigeonpea has 2.0-4.0% soluble sugars (Rao, 1976; Singh et al., 1984a); there are approximately 1.2% sucrose and small amounts of fructose (0.3%) and glucose (0.1%) (Rao & Belavady, 1978). Raffinose and stachyose constitute 12.9 and 15.7% of the soluble sugars, respectively (Singh et al., 1984a).

Lipids

Mahadevappa and Raina (1978) found that pigeonpeas contain 2.0% total lipids of which 50.0% is neutral lipid. The glycolipids and the phospholipids constitute 0.19% and 0.75% on dry weight basis, respectively. Linoleic acid is

the major fatty acid, constituting 56.0% of the total lipids. The major saturated fatty acid is the palmitic acid, which constitutes 20.5% of the total lipids (Mahadevappa & Raina, 1978). Ninety five percent of the lipids are stored in the spherosomes or the lipid containing vesicles of cotyledons (Salunkhe, 1986).

Minerals

Pigeonpea is a good source of dietary minerals such as calcium, phosphorus, magnesium, iron, sulfur, and potassium (Salunkhe, 1986). A study by Singh et al. (1984b) showed that the mature pigeonpeas contain calcium (120 mg/100 gm), magnesium (122 mg/100 gm), phosphorus (290 mg/100 gm), and potassium (1302 mg/100 gm). The mature seeds also contain the trace minerals, iron (4 mg/100 gm), zinc (2 mg/100 gm), copper (1.2 mg/100 gm), and magnesium (1.3 mg/100 gm).

Vitamins

According to Salunkhe (1986), pigeonpea is relatively a good source of water soluble vitamins, especially thiamin, riboflavin, niacin, and the vitamin-like factor, choline. The data on the fat soluble vitamins are limited. According to Sathe et al. (Salunkhe, 1986), there is a large variability in vitamin content of pigeonpeas which can be attributed to the differences in analytical methods and possible differences in the sampling time following harvest

as well as differences in sample preparation procedures.

Antinutritional Factors

Pigeonpea seeds contain several antinutritional and toxic factors such as protease inhibitor (trypsin and chymotrypsin), subtilin inhibitor, phenolic compounds, amylase inhibitor, phytates, cyanogenic compounds, lectins, and saponins (Salunkhe, 1986). Pigeonpea has 17.4-25.1 trypsin inhibitor units/mg protein and 8.9-12.4 chymotrypsin inhibitor units/mg protein (Singh et al., 1990). The presence of protease inhibitors (trypsin and chymotrypsin) reduces the in-vitro protein digestibility of pigeonpea proteins (Singh & Jambunathan, 1981).

The tannin content of the seeds ranges from 0 to 1.26% (Price et al., 1980; Srivastava & Bajpai, 1982). About 27.0 amylase inhibitor units/gm meal are present in pigeonpeas. The cyanide content of pigeonpea varies from 0.45 to 0.70 mg hydrogen cyanide/100gm of seed (Srivastava & Bajpai, 1982). Although the pigeonpea is composed of these various types of antinutritional factors, they are at such low levels that their significance is questioned.

Effect of Processing on the Nutritional Quality of Pigeonpeas

Studies have been conducted on the nutritional quality of processed pigeonpeas. Singh et al. (1990) studied the

effect of cooking on the protein quality of pigeonpeas by conducting rat feeding experiments. The results showed that the protein digestibility was significantly increased by cooking, for both the whole seed and the split seed samples of pigeonpeas (Table 4). In a study by Batra et al. (1986), heat treatment (autoclaving, dry heating, and boiling in water bath) and germination of pigeonpeas decreased trypsin inhibitor activity. Cooking of pigeonpeas decreased the trypsin inhibitor activity from 17.4-25.1 to 8.9-12.4 trypsin inhibitor units/mg protein and completely destroyed the chymotrypsin inhibitor. However, cooking and germination had no significant effect on amino acid composition (Taha, 1987).

Functional Properties

Functional properties of proteins are the physicochemical properties that affect the behavior of proteins in food systems during the preparation, processing, storage, and consumption (Kinsella, 1979; Oshodi & Ekperigin, 1989). Functional properties are used in determining the potential food systems in which a given isolate contributes to the desirable properties (Crenwelge et al., 1974). The important functional properties are protein solubility, water and fat absorption capacities, and emulsion and foaming properties. Data in Table 5

Table 4

Effect of Cooking on Protein Quality of Pigeonpeas

	Whole seeds		Split seeds	
	Raw	Cooked	Raw	Cooked
Protein %				
(dry weight basis)	21.0	20.9	23.1	22.8
TD ¹	60.6	74.9	70.8	85.7
BV ²	64.0	64.5	76.4	62.9
NPU ³	38.8	48.3	54.1	53.9
UP ⁴	8.1	10.1	12.5	12.3

Note. ¹ True Protein Digestibility

² Biological Value

³ Net Protein Utilization (TD X BV / 100)

⁴ Utilizable Protein (%Protein X NPU / 100)

From "Nutritional Quality Evaluation of Newly Developed High Protein Genotypes of Pigeonpea (Cajanus cajan)" by U. Singh, R. Jambunathan, K. Saxena, and N. Subramanyan, 1990, Journal of Science Food and Agriculture, 50, p. 206.

Table 5

Functional Properties of Soy 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
Emulsification	formation and stabilization of fat emulsions	sausages, bologna, soup, cakes
Foaming	forms stable films to entrap gas	whipped toppings, chiffon desserts, angel cakes

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

(Kinsella, 1979) show the functional properties and mode of action of soy proteins and the food systems in which they are applied.

According to Johnson (1970), the acceptability of protein containing products is affected by the functional properties, rather than by the nutritional value. The functional properties of proteins are, in turn, influenced by the immediate environment, such as pH and ionic strength of the food system (Kinsella, 1979).

Protein Solubility

Solubility is one of the important functional properties of food proteins, which is related to protein-water interactions (Kinsella, 1976). The polar amino acid sites are the primary sites for the interaction (Sathe et al., 1984). The solubility characteristics are useful in selecting optimum conditions for extracting the proteins from natural sources (Betschart & Kinsella, 1974). The property of protein solubility is related directly to many important aspects, such as use in beverages, emulsification, and foaming properties (Kinsella, 1976).

The protein solubility is affected by pH, ionic strength, and the protein concentration (Kinsella, 1976). In general, plant proteins have minimum solubility at pH 4.0-5.0, and solubility increases with increasing pH

(Betschart, 1974; Oshodi & Ekperigin, 1989; Sathe et al., 1982a, b). At low or zero ionic strength and at a neutral pH value, protein-water interactions are the greatest, and solubility is maximum for soy proteins (Kinsella, 1979).

Water Absorption Capacity

Water absorption capacity is a functional property that is dependent upon the protein-water interactions (Sathe et al., 1984). The ability of proteins to bind water is an important attribute, especially in the preparation of baked foods (Kinsella, 1979).

The water absorption capacity is influenced by the heat treatment of the proteins and also the pH and the concentration of the system. The heat treatment enhances the water absorption capacity of soy flour (Kinsella, 1976). The water absorption capacity of soy proteins increased six fold when the pH was shifted from 5.0 to 7.0 (Hutton & Campbell, 1977). According to Sathe et al. (1982a), defatting the flour increases the water absorption capacity. As stated by Sathe et al. (1982a), the increase in water absorption capacity may be due to the exposure of water binding sites on side chains of proteins, which are blocked in a lipophilic environment before defatting the flour.

Fat Absorption Capacity

The ability of proteins to bind fat is important for applications, such as meat replacers and extenders, because fat enhances flavor retention (Kinsella, 1976). The hydrophobic proteins lower the surface tension and show superior binding of lipophilic materials, such as flavors and lipids.

Emulsion Properties

The emulsion properties are related to the capacity of proteins to lower the interfacial tension between the hydrophobic and hydrophilic components of food systems. The ability of proteins to aid the formation and stabilization of an emulsion is critical for many applications, i.e., cake batters, coffee whiteners, mayonnaise, salad dressings, and frozen desserts (Kinsella, 1979).

The emulsion capacity of protein decreases with an increase in protein concentration (Lin et al., 1974; Sathe et al., 1982b). The dependence of emulsion capacity on protein concentration is related to the interfacial tension between water and the fat globule (Kinsella, 1979). The globular proteins diffuse slowly because of their molecular size. However, at lower concentrations, they spread easily at the interface of fat and water. As the concentration of

the proteins is increased, the globular protein molecules start to spread slowly at the interface, thereby reducing the emulsion capacity.

The emulsion capacity of soluble proteins depends upon the hydrophilic-lipophilic balance which is affected by the pH (Sathe et al., 1982b). The emulsion capacity at various pH conditions resembles the solubility curve of proteins (Crenwelge et al., 1974), and alkaline pH improves the emulsion capacity (Franzen & Kinsella, 1976; Hutton & Campbell, 1977; Lin et al., 1974).

Emulsion stability of proteins is of concern in food applications such as mayonnaise. The stability of emulsions is minimal near the isoelectric pH, because the proteins aggregate at that pH and destabilize the interfacial membrane (Franzen & Kinsella, 1976). The stability of emulsion is also influenced by the protein fractions. The globular proteins form highly cohesive films which are resistant to mechanical deformation, thereby preventing coalescence (Sathe et al., 1982b).

Foaming Properties

Foaming is an important surfactant property of proteins. It is related to the rate of decrease of surface tension of the air/water interface caused by the adsorption of protein molecules. Protein foams consist of gas bubbles

encapsulated by a liquid film of soluble protein (Kinsella, 1979). The foam capacity is important in several products, such as angel food cakes, sponge cakes, divinity-type confections, candies, meringues, souffles, various whipped toppings, icings, fudges, etc. (Kinsella, 1976).

The foaming properties of proteins depend upon concentration, ionic strength, solubility, pH, etc. (Sathe et al., 1984). Foaming capacity increases with an increase in the concentration of proteins (Sathe & Salunkhe, 1981; Sathe et al., 1982a). The foaming property of proteins improves with the addition of salt at a concentration of 0.6% (w/v) in the slurry. This improvement is attributed to the increased protein solubility (Sathe et al., 1982a). At the isoelectric pH, the foaming capacity is low due to the poor solubility of proteins. In addition, the strong intermolecular forces at the isoelectric pH do not allow protein unfolding, contributing to the low foam formation (Nath & Rao, 1981).

Methodology for Determining the Functionality

There are numerous variations in the methods of determination of functional properties of proteins. The lack of standard procedures of determination of functional properties is a serious problem. Despite the fact that the existing methods are empirical, researchers are currently

using them with necessary modifications to suit various laboratory conditions. The need to develop universally applied methods is emphasized by research recommendations from the National Science Foundation (Regenstein & Regenstein, 1984). According to Regenstein and Regenstein (1984), this will promote better communication between different groups of investigators involved with protein materials.

CHAPTER 2

JOURNAL ARTICLE

FUNCTIONAL PROPERTIES OF PIGEONPEA PROTEIN ISOLATE

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ABSTRACT

The protein isolate was prepared from dehusked split pigeonpeas. The protein solubility profiles of the flour and the isolate were studied at pH range 2.0-12.0. The other functional properties studied were water absorption capacity, fat absorption capacity, emulsion, and foaming properties of the isolate. Pigeonpea protein isolate was rich in protein equalling 88.0% on the dry weight basis. The yield of protein isolate and the recovery of protein from the full fat flour were approximately 20% and 76%, respectively. The isoelectric point of the protein isolate was at pH 4.6. The water absorption capacity was 6.5-7.0 gm H₂O/gm isolate at acid pH and 2.2-4.0 gm H₂O/gm isolate at neutral pH. The fat absorption capacity was 3.2 gm oil/gm isolate. The emulsion and foaming capacities were 118.0 gm oil/gm isolate and 37.39% by volume, respectively, at the neutral pH.

INTRODUCTION

Pigeonpea (Cajanus cajan) is an important legume in semiarid tropical and subtropical countries (Singh et al., 1984a; Yadav, 1983). Pigeonpea is one of the major legumes which contributes significantly to the food protein supply in the countries where it is cultivated. Its economic importance in areas of the world, such as India, East Africa, the Caribbean, and Latin America, is now well recognized.

The protein content of pigeonpea is 15.0-28.0% (Salunkhe, 1986) and rich in lysine (Oshodi and Ekperigin, 1989). However, it is not being utilized as a source of high protein products, i.e., concentrates and isolates. While the composition and the nutritional value of pigeonpea have been the subject of numerous studies (Mahadevappa and Raina, 1978; Modi and Kulkarni, 1976; Oshodi and Ekperigin, 1989; Rao and Subramanian, 1970; Salunkhe, 1986; Singh et al., 1984a, 1990), the literature on the functionality of the proteins is limited. Since there is a continuous increase in demand for protein-rich products by the growing world population, researchers are exploring more and more plant proteins to combat the problem of protein malnutrition in the world. Since the preparation of protein concentrate and isolate is currently

recognized as an effective way to utilize the legume proteins, there is a need to expand data on their functional properties.

Functional properties are the primary factors in determining the utility of proteins as ingredients in various food products (Crenwelge et al., 1974). Systematic determination of functional properties help in predicting the way in which a new protein behaves in specific food systems (Kinsella, 1976). The ultimate success of utilizing plant protein as an ingredient depends largely upon the beneficial qualities which it imparts to the food systems (McWatters and Cherry, 1977). The studies on the functional properties of pigeonpea protein products will contribute to the use of protein products in the food systems. This study was conducted in two phases with the objective of preparing the pigeonpea protein isolate and studying its functional properties at two most desirable pH conditions viz. acidic pH (pH 4.0-5.0) and neutral pH (pH 6.5-7.0).

MATERIALS AND METHODS

Experimental Design. Pigeonpea flour was prepared from the matured dehusked split pigeonpeas. The study was conducted in two phases as described below.

Phase I. The full fat flour was analyzed in triplicate

for proximate composition according to the methods of the Association of Official Analytical Chemists (AOAC, 1984). The flour was defatted and the protein solubility behavior of the flour was determined by the method of Lowry et al. (1951) in order to know the maximum and minimum protein solubility vs. pH.

Prior to establishing the protein solubility curve of the flour, the method of Lowry et al. (1951) for protein estimation was calibrated against the method of Kjeldahl (AOAC, 1984). The regression equation was calculated for converting the Lowry values to the Kjeldahl values.

Phase II. The defatted flour was used to prepare the protein isolate, by the method of How and Morr (1982), based on the pH values obtained in the protein solubility curve of the flour. The protein isolate was prepared in different batches, mixed, and stored in the refrigerator. The isolate was analyzed for the proximate composition.

Protein solubility of the isolate was determined by the method of Lowry et al. (1951) in the pH range of 2.0-12.0. Water absorption capacity, emulsion, and the foaming properties were determined at room temperature, in duplicate, at two pH conditions which are commonly used in the food systems, i.e., pH 4.0-5.0 (acidic foods) and pH 6.5-7.0 (neutral foods). The fat absorption capacity of

protein isolate was determined in triplicate at room temperature.

Calibration Curve. The method of Lowry et al. (1951) was used for protein estimation, because it is less tedious and less time consuming, and it requires a smaller sample (Regenstein and Regenstein, 1984). The Lowry method was calibrated against the Kjeldahl method, because the latter method of protein estimation is recognized as the standard one to estimate proteins.

The protein was extracted from the defatted flour at pH 10.0 (at which the pigeonpea flour has a maximum protein solubility, as determined in the preliminary experiments conducted prior to the actual study), and different volumes of the extract were analyzed for protein by the method of Lowry et al. (1951). The same extract was used to estimate protein (N x 6.25) by Kjeldahl method (AOAC, 1984). A calibration curve was constructed for protein values obtained by the method of Lowry against those obtained from the method of Kjeldahl. The regression coefficient (0.999), the slope (1.41), and the regression constant (2.75) were calculated for the conversion of Lowry values to corresponding Kjeldahl values using the regression equation:

$$Y = 2.75 + 1.41X$$

where, X = Lowry value

Y = Kjeldahl value

Sample and Sample Preparation. Dehusked split pigeonpeas were obtained from a local market in Milpitas, CA. The full fat flour was prepared by grinding the split pigeonpeas in a Straub model 4E grinding mill and passing through a 60 mesh screen. The flour was defatted by extracting it twice with hexane (1:3 v/v) for 8 hr with constant stirring in a hood at room temperature. The defatted flour was air dried for 48 hr to remove the solvent, passed through 60 mesh screen, and stored in the refrigerator.

Several batches of pigeonpea protein isolate were prepared using the method described by How and Morr (1982) for soy proteins. Figure 1 shows the schematic for protein isolate preparation.

Twenty five grams of the defatted flour was suspended in 250 ml distilled water, and the pH was adjusted to 10.0 using 0.2 N NaOH. The suspension was shaken for 30 min in a GCA/Precision Scientific shaker, at the rate of 200 oscillations/min and centrifuged under refrigeration at 1000 X g for 10 min. While the supernatant was saved for further processing, the residue was dispersed in 150 ml distilled water, extracted for 30 min at pH 10.0, and

centrifuged at 1000 X g. The supernatants were combined, and the pH of the combined supernatant was adjusted to 4.3 with 0.2 N HCl. The supernatant was centrifuged at 1000 x g, under refrigeration, for 10 min. The resultant supernatant was discarded, and the precipitate was dispersed in 150 ml distilled water. The pH was adjusted to 10.0 with 0.2 N NaOH and shaken for 30 min after which it was acidified to pH 4.3 with 0.2 N HCl to precipitate the protein. The sample was centrifuged under refrigeration for 10 min at 1000 X g. The supernatant was discarded, and the precipitate was redispersed in 50 ml distilled water and freeze dried. The freeze dried protein isolate was ground in mortar, sieved through 60 mesh screen, and stored in the refrigerator.

Proximate Analysis. Analyses of moisture, crude fat, crude protein, and ash were performed on the full fat flour and the protein isolate according to the methods of the AOAC (1984). The total carbohydrate was calculated by difference.

Protein Solubility. The protein solubility of defatted flour and the isolate was determined by the method of Morr et al. (1985) with modifications. A 500 mg sample was weighed to the nearest 0.1 mg into a 50 ml standard flask, and 30 ml of 0.1 N NaCl solution was added with

continuous stirring to form a dispersion. The pH of the dispersion was adjusted to the desired level ranging from pH 2.0-12.0, using 0.2 N HCl or NaOH. The dispersion was shaken for 45 min on a GCA/Precision Scientific shaker at the rate of 200 oscillations/min. The volume was increased to the 50 ml mark on the flask with the NaCl solution. The dispersion was centrifuged under refrigeration at 3,500 RPM for 30 min and filtered. The amount of protein in the filtrate was determined using the method of Lowry et al. (1951). The protein solubility values were expressed in terms of Kjeldahl, using the regression equation.

Water Absorption Capacity. Water absorption capacity of the isolate was determined at acidic pH (4.0-5.0) and neutral pH (6.5-7.0) using the method described by Sathe et al. (1982a) with modifications. A 1 gm sample was weighed into a graduated centrifuge tube, and 15 ml distilled water was added. The dispersion was stirred for 30 sec in a vortex mixer (Fisher Scientific Model 231). The pH was adjusted using 0.2 N HCl or NaOH, and the volume was made up to 20 ml. The dispersion stood at room temperature for 30 min after which it was centrifuged in Damon/IEC Division clinical centrifuge for 30 min at 7,000 RPM. The supernatant was decanted into a 25 ml graduated cylinder to measure the volume of unabsorbed water. The water

absorption capacity was expressed as gm H₂O absorbed by 1 gm protein isolate.

Fat Absorption Capacity. The method described by Sathe et al. (1982a) was used to determine the fat absorption capacity. One gram of the sample was weighed into a graduated centrifuge tube, and 10 ml of 100% corn oil (food grade) was added to form a dispersion. The dispersion was stirred in the vortex mixer for 30 sec, left at room temperature for 30 min, and centrifuged in a Damon/IEC Division clinical centrifuge for 30 min at 7,000 RPM. The supernatant was decanted into a graduated cylinder to measure the unabsorbed oil. The density of the corn oil used was 0.87 gm/ml. The fat absorption capacity was expressed as the gm fat absorbed by 1 gm protein isolate.

Emulsion Properties. Emulsion capacity and stability were determined at acidic pH (4.0-5.0) and at neutral pH (6.5-7.0) using the method described by Sathe and Salunkhe (1981) with modifications. The dispersion of protein isolate was prepared at 1% concentration in the desired pH range. The dispersion was stirred for 30 sec in an Osterizer blender, at high speed set for stirring. Food grade corn oil (100%) was added to the dispersion continuously with a burette, while blending at high speed. The addition of oil was stopped when there was a drop in

consistency (judged subjectively by the decrease in resistance to blend). The emulsion capacity was expressed as gm oil added to 1 gm protein. The emulsion was poured into a 250 ml graduated cylinder and allowed to stand at room temperature to record the volume of water separated at 0, 8, 12, 16, 24, 48, 72, 96, and 120 hr. This was expressed as the emulsion stability.

Foaming Properties. The foam capacity and stability were determined at acidic pH (4.0-5.0) and at neutral pH (6.5-7.0) by the method described by Sathe et al. (1982a) with modifications. A dispersion of protein isolate at 1% concentration was prepared in the desired pH range. The dispersion was whipped in the Osterizer blender for 5 min at room temperature. The contents (along with the foam) were poured into a 250 ml graduated cylinder. The volumes were recorded before and after whipping. The percentage volume increase was calculated according to Sathe et al. (1982a). The foam stability was determined at 5, 10, 15, 30, and 45 min and 1, 1.5, 2.5, 17, and 18 hr at room temperature.

$$\text{Volume increase(\%)} = \frac{\text{Volume after whipping(ml)} - \text{Volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

Statistical Analysis. The mean and the standard

deviation for the triplicate values obtained in the proximate analysis of each component and for the fat absorption capacity were calculated. The mean was calculated for the duplicate values in the determination of water absorption capacity and emulsion and foaming properties.

RESULTS AND DISCUSSION

The results of the current study are presented in Tables I through IV. The data in Tables V and VI show the comparison of functional properties of pigeonpea protein isolate with those of the other legumes.

Proximate Composition. The results of proximate composition of the full fat flour and the isolate are presented in Table I. The crude protein content ($N \times 6.25$) of 25.6% in the flour on a dry weight basis is within the range of values reported earlier for pigeonpeas (Oshodi and Ekperigin, 1989; Salunkhe, 1986; Taha, 1987). The protein content of the isolate is 88.0% on the dry weight basis. This value is higher than the protein content (82.0%) of rapeseed protein isolate (Soluski et al., 1976) and lower than that (96.0%) of soy protein isolate (Kinsella, 1979). The total carbohydrate content (obtained by difference) of the flour (70.1%) is higher than the values reported in the literature (Oshodi and Ekperigin, 1989; Salunkhe, 1986).

The fat content of the flour is lower than that reported (1.8% on the dry weight basis) in the literature (Oshodi and Ekperigin, 1989; Singh et al., 1990). Although the flour was defatted before preparing the isolate, crude fat content (0.86%) of pigeonpea protein isolate is higher than the reported values of 0.6% and 0.1% for rapeseed protein isolate (Soluski et al., 1976) and soy protein isolate (Kinsella, 1979), respectively.

Protein Recovery and Yield. The protein isolate is light yellow in color and has no detectable odors. The yield of isolate from the full fat flour is approximately 20.0%. The total protein recovered from the full fat flour is calculated to be 76.0%, which is slightly higher than that reported by Filho et al. (1985). They extracted pigeonpea protein by four different methods and obtained the highest yield by aqueous extraction and acid precipitation method, with the addition of sodium hexa meta phosphate at 0.5%.

Protein Solubility. The protein solubility profile of the defatted flour and the isolate is shown in Figure 2. The results show that 67.0% of flour protein is soluble at pH 2.9. The minimum protein solubility occurred at pH 4.3 (isoelectric pH of the flour protein), with 13.3% of protein soluble. There was an increase in solubility from

pH 4.3 to pH 10.0 followed by a decrease above pH 10.0. At pH 10.0, the solubility was greater than 100.0%. This might be due to the presence of polyphenols in the flour, which give darker yellowish brown color at the alkaline pH. It was theorized that the darker color contributed to the higher value when the protein was estimated colorimetrically by the method of Lowry et al. (1951). However, the dark yellowish brown color was not observed in the isolate at alkaline pH, suggesting that, during the process of protein isolation, polyphenols were removed.

The isoelectric pH of the flour protein obtained in this study is slightly lower than the values, pH 5.0 and pH 4.4, reported by Oshodi and Ekperigin (1989) and Taha (1987), respectively. However, the solubility behavior of pigeonpea protein is similar to that reported for wingedbean and soy proteins (Narayana and Rao, 1982).

The protein solubility curve (Figure 2) showed that the isoelectric pH of the pigeonpea protein isolate is at pH 4.6, which is lower than that of the rapeseed protein isolate and higher than that of the soy proteins (Table V). The solubility increased with increase in pH from 4.6 to 10.8, with maximum solubility of 95.0% at pH 10.8. There was no change in solubility from pH 10.8 to 12.0 for the protein isolate.

The protein solubility values (Figure 2) of the isolate are slightly lower than those obtained for the flour at various pH conditions. This suggests that a partial denaturation of protein occurred during the preparation of protein isolate.

Water Absorption Capacity. The results obtained for water absorption capacity are 6.5-7.0 gm/gm at pH 4.0-5.0 and 2.2-4.0 gm/gm at pH 6.5-7.0 (Table II). This is explained on the basis of solubility of proteins at these two pH conditions. At pH 4.0-5.0 (near the isoelectric pH), the quantity of insoluble protein is higher, and, hence, the quantity of water held by the proteins is also higher (Regenstein and Regenstein, 1984). The water absorption capacity of pigeonpea protein isolate is higher than the reported value for pigeonpea flour (about 1.4 gm/gm) (Oshodi and Ekperigin, 1989). At the neutral pH condition, the water absorption capacity of the pigeonpea protein isolate at the neutral pH condition is almost similar to the rapeseed protein isolate (Table V).

Fat Absorption Capacity. The fat absorption capacity of pigeonpea protein isolate was 3.2 gm/gm of protein (Table II), which is much higher than the value reported for pigeonpea flour (0.9 gm/gm) (Oshodi and Ekperigin, 1989). The capacity of pigeonpea protein isolate to hold

fat is also greater than the values reported for soy proteins (Table V) and sunflower protein isolate (2.56 gm/gm) (Ige et al., 1984). Sathe et al. (1982b), postulated that the higher fat absorption capacity of wingedbean proteins is due to the presence of apolar amino acids.

Emulsion Properties. The results of emulsion capacity and the description of emulsion formed at acidic and neutral pH conditions are shown in Table III. The emulsion capacity of pigeonpea protein isolate at pH 6.5-7.0 was 118.0 gm/gm protein. The emulsion formed at the neutral pH was creamy white in color, viscous in consistency, and mayonnaise-like in appearance. At the acid pH (pH 4.0-5.0), pigeonpea protein isolate exhibited poor emulsion capacity. The suspension started to break up exhibiting a curdled appearance when less than 20 ml oil was added to 1% protein solution. Sathe et al. (1982b) suggested that relatively high emulsion capacity of proteins near neutral pH may be helpful in stabilizing colloidal food systems. The emulsion capacity of the pigeonpea protein isolate is greater than the values reported for the rapeseed protein isolate (Table VI). However, wingedbean protein concentrate appears to have a greater emulsion capacity than the pigeonpea protein isolate (Table VI).

The emulsion at neutral pH (6.5-7.0) also had

excellent stability (Figure 3) at room temperature. The emulsion remained stable for 8 hr at room temperature, and the first phase of water separation occurred at 12 hr of standing. There was an increase in volume of water that separated with an increase in time. The emulsion stability at the acidic pH was not monitored because, as soon as the emulsion was poured into a graduated cylinder, more than 50% of water separated.

Foaming Properties. The results obtained for foaming capacity are presented in Table IV. The foaming capacity of pigeonpea protein is 4 times higher at pH 6.5-7.0 (37.4%) than at pH 4.0-5.0 (9.13%). The foam formed at the neutral pH was thick and closely packed. The lower foaming capacity observed at pH 4.0-5.0 (near isoelectric point) is due to the low solubility of proteins at that pH. Another factor which contributed to lower foaming capacity at this pH is the strong inter molecular forces which do not allow protein unfolding and thus impair the protein film formation at the interface (Nath and Rao, 1981). The foam capacity of pigeonpea proteins is lower than the reported values for soy proteins and rapeseed proteins (Table VI), and this may be due to the higher globulin fractions in pigeonpea proteins. The globulin fraction of proteins are difficult to surface denature and, hence, result in lower

foaming capacity (Sathe et al., 1982b).

The results of foam stability of pigeonpea protein isolate at room temperature are presented in Figure 4. The stability of the foam was high at pH 6.5-7.0, with no change in the percent foam volume after standing 15 min at room temperature. The stability of the foam decreased with time, and, at the end of 18 hr, the foam volume recorded was only about 0.9%.

The foam stability at pH 4.0-5.0 was low when compared at the neutral pH. The volume of foam was reduced to 50.0% of the initial volume after 5 min of standing at room temperature. Within 1.5 hr, the foam volume was reduced to 0.9%. These findings are similar to those for the soy protein which show poor foaming properties near the isoelectric pH due to lower protein solubility (Kinsella, 1979).

CONCLUSION

The yield of pigeonpea protein isolate from the full fat flour is about 20% and the total protein recovered from the full fat flour is about 76%. The isolate is light yellow in color and has no detectable odors which are desirable characteristics in food applications.

The protein solubility curve showed that the protein of the isolate is less soluble than the flour at various pH

conditions, suggesting that a partial denaturation of protein occurred during the isolate preparation. The solubility behavior of the pigeonpea proteins is similar to that of the soy and winged bean proteins (Narayana and Rao, 1982). The water absorption capacity of pigeonpea proteins at the neutral pH is almost similar to the rapeseed protein isolate (Table V). The fat absorption capacity of the protein isolate is high compared to the other legume proteins (Ige et al., 1984; Kinsella, 1979; Narayana and Rao, 1982). The formation of creamy stable emulsion with the isolate at room temperature suggests that the pigeonpea protein isolate may be useful in producing mayonnaise-like products. Although foam capacity of pigeonpea protein isolate is about four times higher at neutral pH than at acid pH, in general, the percent foam capacity values are lower than those for the soy isolate (Kinsella, 1979).

Figure 1. Schematic for protein isolate preparation.

Figure 2. Effect of pH on the protein solubility of the pigeonpea flour and protein isolate in 0.1 M NaCl solution, at room temperature.

Figure 3. Emulsion stability of pigeonpea protein isolate in neutral pH (6.5-7.0) condition at room temperature. Average values of duplicate measurements are plotted.

Figure 4. Foam stability of pigeonpea protein isolate at acid (pH 4.0-5.0) and neutral (pH 6.5-7.0) pH conditions at room temperature. Average values of duplicate measurements are plotted.

Table I. Proximate Composition of Pigeonpea Flour and Isolate

composition %	full flat flour ^a		protein isolate ^a	
	based on		based on	
	wet weight	dry weight	wet weight	dry weight
moisture	7.9±0.28	-	1.6±0.03	-
crude protein (<u>N</u> x 6.25)	22.6±0.19	25.6±0.21	86.6±0.04	88.0±0.04
crude fat	1.6±0.02	1.8±0.02	0.86±0.05	0.88±0.04
ash	3.4±0.08	3.7±0.09	0.97±0.03	0.98±0.02
carbohydrates (by difference)	64.6±0.15	70.1±0.17	9.9±0.10	10.1±0.02

^a mean ± standard deviation

Table II. Water and Fat Absorption Capacities of Pigeonpea Protein Isolate

functional property	gm/gm of protein isolate
water absorption capacity	
pH 4.0-5.0	6.5-7.0
pH 6.5-7.0	2.2-4.0
fat absorption capacity ^a	3.2 $\pm$ 0.04

^a mean $\pm$ standard deviation

Table III. Emulsion Capacity of Pigeonpea Protein Isolate
at Acid and Neutral pH Conditions

pH	emulsion capacity (gm/gm) ^a	volume of emulsion (ml) ^a	description
4.0-5.0	< 20	120.0	thin, pourable
6.5-7.0	118.0	248.0	viscous, mayonnaise-like

^a mean of the duplicate measurements

Table IV. Foaming Capacity of Pigeonpea Protein Isolate at Acid and Neutral pH Conditions

pH	% volume increase ^a	description
4.0-5.0	9.13	loosely packed, bubble size not uniform
6.5-7.0	37.39	closely packed, small bubbles, uniform size

^a mean of the duplicate measurements

Table V. Comparison of Protein Solubility, Water Absorption Capacity (WAC) and Fat Absorption Capacity (FAC) of Various Legume Proteins

Functional property	pigeonpea		rapeseed	soy	wingedbean
	F	I	I	C	C
Protein solubility					
Isoelectric pH	4.3 ¹	4.6 ¹	6.0 ²	4.0 ²	4.0 ³
WAC (gm/gm)					
pH 4.0-5.0	-	6.5-7.0 ¹	-	3.3 ²	-
pH 6.5-7.0	-	2.2-4.0 ¹	3.1 ²	-	-
pH not given	1.4 ⁴	-	-	-	3.5 ³
FAC (gm/gm)	0.9 ⁴	3.2 ¹	3.8 ²	2.0 ²	4.0 ³

F - Flour, I - Isolate, C - Concentrate. ¹ Results of the current study. ² Soluski et al. (1976). ³ Sathe et al. (1982b). ⁴ Oshodi & Ekperigin (1989).

Table VI. Comparison of Emulsion Capacity (EC) and Foaming Capacity (FC) of Various Legume Proteins

Functional property	pigeonpea		rapeseed	soy	wingedbean
	F	I	I	C	C
EC (gm/gm)					
pH 4.0-5.0	-	<20.0 ¹	-	0.1 ²	-
pH 6.5-7.0	-	118.0 ¹	0.4 ²	-	-
pH not given	0.5 ⁴	-	-	-	222.2 ³
FC (% volume increase)					
pH 4.0-5.0	-	<9.1 ¹	-	-	-
pH 6.5-7.0	-	37.4 ¹	-	-	-
pH not given	68.4 ⁴	-	365.0 ²	133.5 ²	36.0 ³

F - Flour, I - Isolate, C - Concentrate. ¹ Results of the current study. ² Soluski et al. (1976). ³ Sathe et al. (1982b). ⁴ Oshodi & Ekperigin (1989).

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Functional properties of the pigeonpea protein isolate were determined at the two most commonly used pH conditions in food systems, i.e., acidic pH (4.0-5.0) and neutral pH (6.5-7.0). The proximate analyses of the original sample (full fat flour) and the isolate were performed using the standard methods of American Association of Analytical Chemists (1984). The method of Lowry et al. (1951) was used for protein estimation, because it was less tedious and less time consuming, and it required a smaller sample (Regenstein and Regenstein, 1984). The Lowry method was calibrated against the Kjeldahl method, because the latter method of protein estimation is generally recognized as the standard one to estimate proteins. The calibration curve, showing the relationship between the protein contents as determined by the methods of Lowry et al. (1951) and Kjeldahl (AOAC, 1984) is presented in the Appendix I.

The solubility behaviors of the defatted flour and the isolate were determined in the pH range of 2.0-12.0, according to the method of Morr et al. (1985). The water absorption capacity, the fat absorption capacity, and the foaming properties of the protein isolate were studied

according to the methods of Sathe et al. (1982a), with necessary modifications. The emulsion properties of the isolate were studied according to the method described by Sathe and Salunkhe (1981).

The proximate analysis of the full fat flour and the isolate showed that the protein contents were 25.6% and 88.0% in the flour and the isolate, respectively. The fat content was low in pigeonpea flour. The yield of protein isolate from the full fat flour was approximately 20% and the total protein recovered from the full fat flour was about 76%.

The solubility of the isolate was lower than that of the flour at various pH conditions, suggesting that a partial denaturation of protein occurred during the isolate preparation. The isoelectric pH was 4.3 for the flour and 4.6 for the isolate. The maximum solubility was at pH 10.0 for the flour and pH 10.8 for the isolate. The water absorption capacity of the isolate was higher at the acidic pH than at the neutral pH. The values were higher than the reported value for the pigeonpea flour (Oshodi & Ekperigin, 1989). The fat absorption capacity of the pigeonpea protein isolate was higher than other legume proteins, such as soybean (Table V) and sunflower (Ige et al., 1984).

The emulsion capacity of pigeonpea protein isolate was

higher than that of the soy proteins. It formed a creamy, viscous, mayonnaise-like emulsion near the neutral pH, which was stable for 8 hr at room temperature. The emulsion formed at the acidic pH had a curdled appearance and was thin and poury in consistency. The stability at acid pH was not evaluated because more than 50% of water separated when the emulsion was poured into the measuring cylinder.

The foam formed at the neutral pH was thick and closely packed. It remained stable for 15 min at room temperature. However, the foam formed at the acidic pH was not as stable, and the volume of foam was reduced by about 50% of the original after standing for 5 min at room temperature. Pigeonpea proteins are similar in this respect to soy proteins which have poor foaming properties at the isoelectric pH (Kinsella, 1979).

Recommendations

The preparation and use of pigeonpea protein isolate is highly recommended in the countries where it is grown abundantly. Further study needs to be conducted to determine the economical aspects in terms of percentage yield of pigeonpea protein isolate from the full fat flour. Further research on the other functional properties of pigeonpea protein isolate, such as gelation, viscosity, etc., is recommended. There is also a need to determine

whether heat has any effect on protein denaturation, thereby affecting its functionality. Furthermore, the effect of the method of protein isolation on the functional properties of pigeonpea proteins needs to be studied. Since the pigeonpea proteins showed excellent emulsion properties at neutral pH, further research could be done to determine the effect of concentration, ionic strength, and the ratio of oil to water on the emulsion properties. Further research is recommended to determine the functional properties of pigeonpea protein isolate in the actual food systems.

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APPENDIX I

The Relationship between Pigeonpea Protein Contents as Determined by the Methods of Lowry et al. (1951) and Kjeldahl (AOAC, 1984).