EXTRACTION AND CHARACTERIZATION OF PROTEIN FROM FRUIT BY PRODUCTS OF JACKFRUIT, MANGO AND LITCHI SEEDS

A THESIS

BY

ABDIRIZAK AHMED GULED

STUDENT ID. 1805174

SESSION: 2018-2019

SEMESTER: January-June, 2019

MASTER OF SCIENCE (MS)

IN

FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

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Hajee Mohammad Danesh Science and Technology University
Dinajpur-5200, Bangladesh

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Dedicated to My

Beloved Parents, Affectionate Uncles,

Brother and Sisters

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The Author

ABSTRACT

Extraction of protein from fruit by products and characterization their functional properties were analyzed in this study. Protein extracted by using distilled water, sodium hydroxide and hydrochloric acid. All protein isolated from fruit by product such mango, jackfruit and litchi seed Prepared via NaOH extraction which seed protein content was resulted 28.23%, 43.12% and 36.96%, respectively. The highest protein content was obtained from jackfruit seed protein isolated. Instead the moisture content was investigated and obtained the protein extracted from mango seed, jackfruit and litchi seed were resulted in 3.68%, 15.93%, 16.60%, respectively. Where ash content of three different fruits by-product of MSP, JSP and LSP protein isolate shown as 1.36%, 1.23% and 2.07%, respectively. Furthermore, litchi seed protein isolate contained little bit high amount of ash then the other seed. In this study shown high variation of fat content among different three by-products of mango, jackfruit and litchi seed protein isolate, resulted 14%, 2% and 1%, respectively. Solubility of a protein is one of the critical functional attributes required for its use as a food ingredient. The resulted the protein solubility of different protein seeds extraction of (MSP, JSP, LSP), at different pH levels between 2 and 12. The minimum solubility of mango, jackfruit and litchi seeds protein were found 10.91%, 7.14% and 18.68%, respectively at pH 4, while the solubility were showed in similar results at pH 2. The solubility of three different fruit by-product seeds isolated protein increased from pH 6 to pH 12, and the maximum solubility of 27.27%, 21.42% and 20.75%, respectively was obtained at pH 12. The water absorption capacity of protein seed extraction were 1.48, 2.99 and 2.266 ml H_2O/g while fat absorption capacity was resulted 1.393 ml oil/g, 1.488 ml oil/g and 1.528 ml oil/g respectively. The overall results showed that by-product protein extraction might be used protein supplement.

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ABBREVIATION

% = Percentage

°C = Centigrade

A/W = Air in Water

DDCF = Dehulled Defatted Cowpea Flour

DW = Distill Water

FAO =Food and Agriculture Organization

FC = Foam Capacity

FS = Foam Stability

G = Gram

g/ml = Grams Per Milliliter

H_2O = Dihydrogen Monoxide

JSP = Jackfruit Seed Protein

LSP = Litchi Seed Protein

MSK = Mango Seed Kernel

MSP = Mango Seed Protein

O/W = Oil in Water

OAC = Oil Absorption Capacity

PH = Potential Hydrogen

RCF = Relative Centrifugal Force

SHMP = Sodium Hexametaphosphate

TPI = Total Protein Isolation

WAC = Water Absorption Capacity

WCF = Whole Cowpea Flour

CHAPTER I

INTRODUCTION

Fruits from the temperate zone are usually distinguished by a large edible portion and intensity aggregate of waste material such as peels, seeds and stones. In contrast, notably higher ratios of by-products arise from tropical and subtropical fruit processing. Due to increasing production, disposal represents a growing problem since the plant material is usually prone to microbial spoilage, thus limiting further exploitation. On the other hand, costs of drying, storage and shipment of by-products are economically limiting factors (Lowe and Buckmaster, 1995).

Protein isolate process has been developed based on the extraction, purification and recovery of protein seeds. (Berk, 1992). A typical procedure for protein isolate production commonly consists of three main steps: (1) protein extraction, (2) protein precipitation, and (3) drying of the protein precipitate. Especially the first two steps (extraction, precipitation), which are directly linked to protein solubility, are significant for the efficient isolation of proteins. For this reason the controlling factors that affect protein solubility have been studied in processes using various protein sources. The economic feasibility of protein isolation process depends on the optimization of each one of these steps (Liadakis *et al*., 1995; Park and Bean, 2003; Salcedo-Cha ´vez *et al.*, 2002; Tzia, 2003).

Protein is vital for human life as it provides essential nutrients required for maintaining normal body growth and development. The escalating population worldwide has resulted in a shortage of food in some regions of the world .moreover, poverty and improper distribution to food security. The conditions require vigorous planning and setting new goals and objectives as the government level, thus ensuring consistency in food availability. The developing world is also facing the menace of protein energy malnutrition due to an insufficiency supply of proteins or intake of proteins with poor nutritional value (Arshad and Shafqat, 2012).

The role for food proteins in human nutrition is substantial, while according to modern nutrition recommendations the supply of proteins in human diet should rely mostly on vegetable proteins, i.e., legumes, oilseeds, etc., then on those from animal sources, i.e., meat. Because the amounts of proteins needed to cover the nutritional needs of the world

population are continuously increasing, the exploration and detecting of new protein sources are of interest. In addition to their nutritional value, proteins offer great potential as functional food ingredients providing useful properties when incorporated into foods. In order to utilize a by- product as a protein source it should both present high protein content and protein value (quality) based on well-balanced essential amino acids, several protein products (flours, concentrates, or isolates), depending on their protein content, can be introduced to food products in order to improve their nutritional value as well as their sensory and functional properties (Thebaudin *et al.,* 1997).

Jackfruit (*Artocarpus heterophyllus* L.), locally known in Bangledhesh as 'Kathal', belongs to the family Moraceae (Bose, 1985; Bhattacherjee, 1986). The jackfruit is indigenous to India and is widely grown in Bangladesh, Burma, Sri Lanka, Malaysia, Indonesia, Philippines, Brazil and other countries (Narasimham, 1990). Jackfruits are composed of several berries of yellow pulp and brown seeds encased in a hard shell and are rich in carbohydrates, complex B vitamins and minerals. However, only 15–20% of the fruit is used as food, which can be cooked, baked or roasted on coals (Silva *et al.* 2007).

The mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world, thanks to its pleasant taste and aroma and high nutritional value (Ibarra *et al.* 2015). It is rich in water, sugars, fibre, minerals, vitamins, and antioxidants (Tharanathan *et al.* 2006). Mango kernels contain fats in the range of 8.5–10.4% along with protein, ash, carbohydrate contents, i.e., 4.76–6.70%, 1.74–2.26%, and 71.90–76.28%, respectively, the protein content in mango kernel is higher than some other fruits (Saddique *et al*., 2014).

Litchi (*Litchi chinensis* Sonn.) is a member of the Sapindaceae family, which is a tropical to subtropical fruit originated from China (Zhao *et al.*, 2007). The fruit manifest desirable flavor and high sugar content with many minerals and vitamins. It can be eaten fresh or as a processed product. Litchi fruit has gained popularity as an exotic fruit in temperate regions and are prized on the international market. Litchi by-products mainly consist of pericarp and seed, which are good sources of bioactive substances, like epicatechin, gallic acid and procyanidins (Prasad *et al.,* 2009). Accordingly, the aim of this work was to extraction protein from mango, jackfruit and litchi seed with

characterization of their functional properties. Therefore the specific objective of this study were as follows:

1. To extract the protein from fruit by-product of jackfruit, mango and litchi seeds.
2. To analyse the physico-chemicals and functional characteristics of protein extraction.

CHAPTER II

REVIEW OF LITERATURE

2.1 By-products

Laufenberg *et al.* (2003). Fruit, vegetable, and oilseed processing result in various amounts of by-products depending on the raw material. The baudin *et al.* (1997). The fruit and vegetable have high amounts of waste materials such as, peels, seeds and oilseed meals also by-product are rich in dietary fibers, some of contain amounts colorants, antioxidant compound or other substance with positive health effect.

Mirzaei *et al.* (2008), Boucque *et al*, (1988). Grape marc is the residue of the wine industry after the juice has been pressed out. The dried marc consists of 40% seeds and 60% pulp. After separating the pulp, the seeds are dried and cleaned. Oil is extracted with hexane, leaving about 85% grape-seed oil meal.

Martinez *et al*, (1980). The extraction of the juice from citrus fruits gives us citrus pulp as residue. Citrus pulp consists of 60- 65% peels, 30-35% segment pulp and 0-10% seeds. On average citrus pulp represents 60% of the fresh weight with a mean DM of 19.7%, but the residue can range between 49 and 69% of the initial weight. There has some interest in the separation of the seeds. Citrus seed meal is left after the oil has been extracted from the citrus seeds.

Denek *et al.* (2006). The manufacture of tomato juice and puree provides peels and seeds as residue. They account for about 4.5% of the fresh weight: 3% peels and 1.5% seeds. In some countries, seeds are used for oil production. Suleria *et al.* (2015). By-products and wastes can be categorized as (1) agricultural harvest generated by-products, (2) postharvest by-products, and (3) food-processing by-products and wastes.

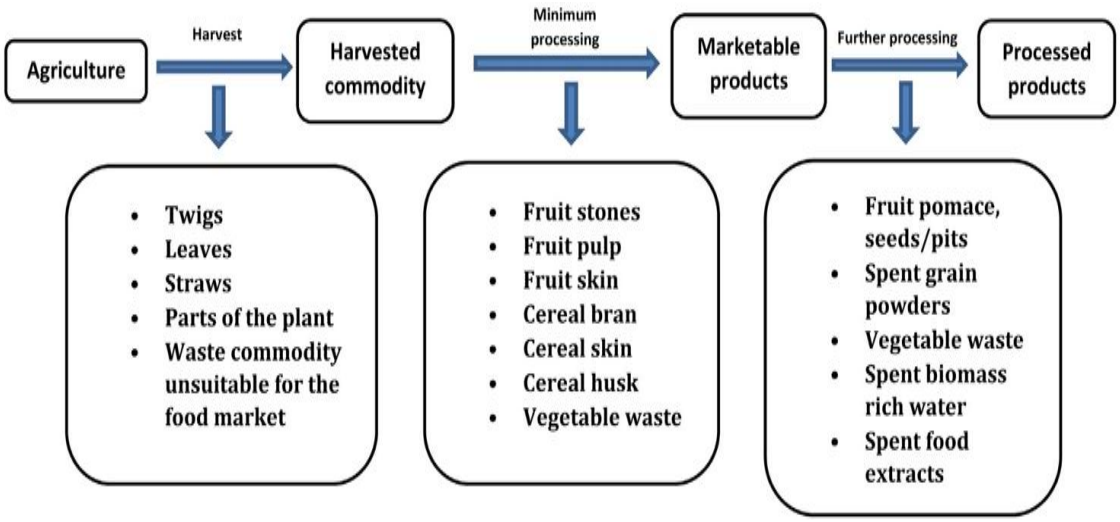

Figure 1: Showed by-products and waste produced during agricultural, postharvest, and food industry unit operations

2.2 Extraction and isolation of protein

Makri *et al.* (2005). This study was developed, Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in mixture with polysaccharides. The production of plant protein isolates and especially legumes is of growing interest to industry, because of the increasing application of plant protein in food and non-food markets. The use of plant protein isolates in foods as functional ingredients to improve the texture, the nutritional quality of the product or for economical reasons is much extended. In spite of the relatively high cost of protein from meat, eggs and dairy products, they are consumed in an amount approximately 10 times greater than legume proteins, even though the later are abundant in protein. The objective of our investigation was to characterize some of the functional properties of legume seed protein isolates from lupin, pea and broad beans, prepared by isoelectric precipitation and ultrafiltration, by preparing emulsions, foams and gels. In our study we use native (as could be) protein isolates, as emulsifiers and stabilizers, in admixture with polysaccharides, in order to create a rigid and viscoelastic film around the oil or air droplets and/or for creation of network structure, with desirable texture for the benefits of the consumer. In all cases emulsions stabilized with proteins precipitated at their isoelectric point, even if they have similar initial droplet size distribution with the ultra filtration one illustrated greater stability throughout the storage time. The addition of incompatible xanthan gum enhances protein adsorption at air-in-water a/w and oil-in-

water o/w interfaces. In this case, emulsions and foams increase their stability. This may be due to xanthan gum's ability to increase the viscosity of the continuous phase. But also to its effect on protein adsorption at the interface. The emulsion stability for all isolates studied appeared more or less the same. The better foaming quality is exhibited by pea, followed by broad bean and lupin. The addition of NaCl leads to the destabilization of the systems, at the used concentrations. The presence of albumins in ultrafiltration isolates leads to a better foamability, but lowers slightly the stability. Lupin forms gels with the greater fracture properties among all three legumes, for all combinations studied. A strong gel (probably 'filled type') is established only with the addition of a mixture of polysaccharides and after 10 days of ageing. The desired texture of the final product will determine the preferred combination according to its profile analysis parameters.

Chavan *et al.* (2001). This experiment was conducted Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.) Protein isolates of beach pea were prepared using sodium hydroxide (NaOH) and sodium hexametaphosphate (SHMP). Functional properties of the isolates so prepared were investigated and compared with those of other pea samples. Protein isolate of beach pea, prepared via NaOH extraction, had a protein content of 86.6%, while SHMP-extracted isolates contained 85.1%protein. Corresponding values for NaOH-and SHMP-extracted green pea and grass pea were 90.6,89.9,90.6 and 88.3%, respectively. Sulphur-containing amino acids were more prevalent in SHMP-extracted beach pea and green pea, while they were higher in NaOH-extracted grass pea. Tryptophan content was higher in NaOH-extracted than SHMP-extracted isolates. The predicted biological value and protein efficiency ratio of beach pea protein isolates indicated the high quality of products so prepared. Beachpea protein isolates exhibited a minimum solubility at pH 4.5. The pH and NaCl concentration effectively changed the functional properties of protein isolates. Beach pea protein isolates(NaOH-and SHMP-extracted) had in-vitro digestibility of 80.6to82.6% for pepsin-trypsin and 78.6 to 79.2% for pepsin-pancreatic.

Coffman *et al.* (1977). Department of Food Science and Technology, UP Los Banos, College of Agriculture, College, Laguna, Philippines. This studied was conducted Functional properties and amino acid content of a protein isolate from mung bean flour. A protein isolate was prepared from mung bean flour by extraction with 0.001 N NaOH, precipitation at pH 4.5, neutralization of the dispersed precipitate to pH 6.8-7.0, and

subsequent freeze drying. The isolate's amino acid composition was determined and found to be similar to that of mung bean flour except for cystine which was destroyed during isolate preparation. The following properties of the protein isolate were investigated: nitrogen solubility, buffer capacity, formability, gelation. Except for buffer capacity, the isolate demonstrated good functional abilities in simple systems under laboratory conditions.

Eromosele *et al.* (2008) Biochemistry Department, University of Agriculture, Abeokuta, Ogun State, Nigeria, this experiment was investigated Extractability of African yam bean (Sphenostylisstenocarpa) protein in acid, salt and alkaline aqueous media. Protein extractability from defatted Africa yam been (Sphenostylisstenocarpa) was studied under various conditions: solid/solvent ratio, time, pH, and salt. Extractable protein from S. stenocarpa was strongly dependent on all these factors. Maximum extractable protein was obtained after 2 h extraction time; the solid to solvent ratio in the range of 1:20–1:50 gave maximum protein extractability. The pH corresponding to maximum and minimum extractable proteins were 10 and 5, respectively, but addition of NaCl changed this slightly. Extractable protein of 92%, 88% and 84% were obtained in aqueous concentration of 0.01MNa2SO4, 1.5MNaCl and 0.01MNaOH, respectively while other concentrations gave lower extractability. These salt concentrations, i.e. 0.01MNa2SO4 and 1.5 MNaCl gave slightly lower extractable protein under alkaline condition. S. stenocarpa flour has a higher buffer capacity in acid medium than in alkaline medium.

Lqari *et al.* (2002). This experiment was conducted Lupinusangustifolius protein isolates: chemical composition, functional properties and protein characterization. the result showing us Two types of protein isolates were prepared from Lupinusangustifolius defatted flour by alkaline extraction, with (Isolate B) and without (Isolate A) sodium sulphite, and acid precipitation of proteins at the isoelectric point (IEP 4.3). Chemical composition, main functional properties and protein composition of L. angustifolius defatted flour and protein isolates were determined. Isolate A and B have 93.9 and 84.6% protein content, respectively, and had a balanced composition of essential amino acids, with respect to the FAO pattern except for lysine.

2.3 Proximate composition

2.3.1 Moisture Content

Abdalla *et al.* (2007).this experiment was carryout Egyptian mango by-product 1. Compositional quality of mango seed kernel.Egyptian mango seeds were collected as wastes from local fruit processing units and the kernels were separated and dried. This study was carried out on mango seed kernels to clarify their proximate composition. The moisture content of fresh mango seed samples was on average 50.7% while the moisture content of dried MSK powder samples was on average 8.5%.

Kingsly *et al.* (2006). this experiment was reached Moisture dependent physical properties of dried pomegranate seeds (Anardana). The moisture dependent physical properties of dried pomegranate seeds (anardana) in the moisture range of 6–18.13% (w.b.) were determined. The size and mass increased from 3.5 mm to 4.4 mm and 28.98 g to 32.58 g, respectively, when the moisture content increased from 6% to 18.13%.

Oclooetl (2010). this experiment was observed Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds at Biotechnology and Nuclear Agriculture Research Institute, Ghana this experiment was resulted Moisture provides a measure of the water content of the seed flour and for that matter its total solid content. It is also an index of storage stability of the flour. The moisture content of the seed flour was 6.09 %. The lower the moisture content of flour, the better its shelf stability and hence the quality. Moisture contents of flour generally is depended upon the duration of the drying process.

de Moraes Crizel *et al.* (2013). this experiment was investigated Food Science Department, Federal University of Rio Grande do Sul, Bento Gonçalves. Dietary fiber from orange by-products as a potential fat replacer. Two samples of orange fiber were produced: F1 (peel, pulp and seeds) and F2 (peel). Shows the composition of the samples moisture contents (7.1-7.9 g/ 100 g).

Baümler *et al.* (2006). Moisture dependent physical and compression properties of safflower seed. The objective of this study was to investigate the effect of moisture content on some physical properties and fracture resistance of the safflower seeds typically cultivated in Argentina. The safflower seeds have an oil content of 43 ± 3.6%

dry basis (d.b.), 37% (d.b.) hull contents and the initial moisture content of the safflower seeds was 6.9% (d.b.).

Konak *et al.*, (2002). Physical Properties of Chick Pea Seeds. This experiment shaw us different physical properties of chick pea seeds were evaluated as functions of moisture content. The average length, width, thickness, the geometric mean diameter, unit mass and volume of seed were 9.342mm, 7.722 mm, 7.752 mm, 8.358 mm, 0.324 g and 0.238 cm3, respectively, at a moisture content of 5.2% d.b. Studies on rewetted seed showed that as moisture content increased from 5.2 to 16.5% d.b.

2.3.2 Ash content

Kumar *et al.* (1988). The ash content is the organic residue remaining after the organic matter has been burnt away. This experiment was researched proximate composition of jackfruit seed, found that ash content of two varieties of jackfruit seed was 1.27 and 1.16%.

Shimelis and Rakshit (2005). This study was performed Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. were studied for their proximate composition of ash content showing us 2.86–4.26 g/100 g.

Martínez *et al.* (2012). This study was conducted Chemical, technological and in vitro antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. This experiment was developed the ash content a mango its was 4.2 g/100g, were guava ash 2.4 and also pineapple ash was 4.5, were passion fruit ash was 5.0 g/100g.

Onimawo *et al.* (2003). This study was observed physicochemical and nutrient evaluations of African bush mango seeds and pulp were conducted. The seeds contained 2.26% mineral ash.

2.3.3 Fat content

Tulyathan *et al.* (2002). This study was carryout some physicochemical properties of jackfruit (Art ocarpushetero phyllus lam) seed flour and starch. Were found jackfruit seed flour contained 0.50-0.99%. Ocloo *et al.* (2010). this research was conducted phsico-chemical, functional and pasting characteristics of flour produced from jackfruits

(*Artocarpushetero ophyllus*) seeds, were resulted 17.2% fat content in jackfruit seed flour.

Ajayi *et al.* (2006). This experiment was observed Oil content and fatty acid composition of some underutilized legumes from Nigeria, they were resulted these Three underutilized legumes from Nigeria, *Brachystegia eurycoma*, *Tamarindus indica* and *Mucuna flagellipes*, have been subjected to standard analytical techniques in order to evaluate proximate composition, physicochemical properties and contents of nutritional valuable elements and fatty acids of the seeds and oils. The proximate analysis indicated that the oil content was 5.87, 7.20 and 3.77 g/100 g for *B. eurycoma, T. indica and M. flagellipes*, respectively.

Shimelis and Rakshit (2005). This study was conducted performed Proximate composition and physico-chemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. The nutrition related parameters studied were dry bean Crude fat ranged 1.27–3.02 g/100 g,

2.4 Functional properties

2.4.1 Bulk density

Ogunwolu *et al.* (2009). This experiment was conducted, Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut bulk density of defatted cashew nut powder (0.48 g/ml) was found to be higher than that of cashew nut protein concentrate (0.31 g/ml), which was in turn higher than that of cashew nut protein isolate (0.25 g/ml.

F.C.K. Ocloo *et al.* (2010). This study was carry out by Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds, the jackfruit seed Bulk density is depended upon the particle size of the samples. The value obtained from the study was 0.80 g/ml.

Monteiro and Prakash (1994). This research was evaluated by Functional Properties of Homogeneous Protein Fractions from Peanut *(Arachis hypogaea* L.), they conducted Department of Protein Technology, Central Food Technological Research Institute, Mysore, India so they resulted Bulk densities of peanut total protein, arachin, conarachin I, and conarachin I1 were found to be 0.253, 0.312, 0.080, and 0.084 g/mL, respectively.

Wani *et al.* (2017). This experiment was observed by Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. So they resulted Pulses are an essential component of our diet especially in developing world, information on their physical properties is needed for designing the machines, while cooking quality is important for consumer acceptance. Four kidney bean cultivars were evaluated for their composition, physical, cooking and textural properties. And the Bulk density varied from 0.78 to 0.81 g/mL.

Ulloa *et al.* (2011). A study was carry out by Physicochemical and functional properties of a protein isolate produced from safflower (*Carthamus tinctorius* L.) meal by ultrafiltration and the result showing us the bulk density of safflower protein isolate obtained by ultrafiltration was 0.27 g ml.

Ogunwolu *et al.* (2009). This experiment was conducted the Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut and they resulted the bulk density of defatted cashew nut powder (0.48 g/ml) was found to be higher than that of cashew nut protein concentrate (0.31 g/ml), which was in turn higher than that of cashew nut protein isolate (0.25 g/ml).

Khalid *et al.* (2003). This experiment was collected the Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration they resulted the bulk density of 0.71 gm/ml.

2.4.2 Water absorption

Abbey and Ibeh (1988). This data was collected by functional Properties of Raw and Heat Processed Cowpea (*Yignaun guicukta*, Walp) Flour. the Results of Water absorption by the raw and heat processed cowpea flours was 2.4 and 3.6 g/ml, respectively, this result suggested that heat treatment affected the water adsorption capacity of the native proteins.

Ulloa *et al.* (2011). This experiment was conducted by Physicochemical and functional properties of a protein isolate produced from safflower (*Carthamus tinctorius* L.) meal by ultrafiltration they was resulted The safflower protein isolate in this study had a water absorption capacity of 2.22 mL $H_2 O_2$ /g protein .

Nassar (2008). This experiment was carry out by Chemical Composition and Functional

Properties of Prickly Pear (*Opuntiaficus indica*) Seeds Flour and Protein Concentrate they resulted was PPS flour and its protein concentrate had high water absorption 4.71 and 3.16g water/g seed flour.

2.4.3 Fat absorption

Sze-Tao and Sathe (2000). This study was showed that Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate it was showed us the two different sample fat absorption and its API registered a higher oil absorption capacity when compared to that of SPI (3.55 and 2.926 g of oil/g protein isolate, dwb; respectively). The high oil absorption capacity of API, despite its high solubility in water, suggests the presence of an appreciable number of hydrophobic residues on protein surface. Further studies on API using hydrophobic probes will be required to verify the presence of hydro- phobic residues on protein surface.

Monteiro and Prakash (1994). This experiment was conducted mysore india by Functional Properties of Homogeneous Protein Fractions from Peanut *(Arachis hypogaea* L.) and they found this result fat absorption capacity values obtained for total protein, arachin, conarachin I, and conarachin II were 1.22, 1.28, 1.26, and 1.29 g of oil/g of protein respectively.

Khalid *et al.* (2003). This experiment was collected the Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration fat absorption was resulted 1.50 ml oil/g protein .

2.4.4 Foaming properties

Nassar (2008). This study was developed by Egypt and its Chemical Composition and Functional Properties of Prickly Pear (*Opuntia ficusindica*) Seeds Flour and Protein Concentrate, the foam capacity (FC) of PPS flour and protein concentrate is shown The lowest FC (30 and 27%) was obtained at pH 4.5 (isoelectric point of protein); at this point the molecules are in more compact from than other pH values. FC significantly increased, especially at pH 8 and 10 reaching 84 and 96; 97 and 103% for both PPS flour and protein concentrate.

The effect of pH and time on the foam stability of the PPS flour and protein concentrate is shown in Regardless of the mixture pH, foam stability gradually decreased with time. At pHs 4.5 and 6 the foam stability gradually decreased and reached to 11 and 12% when the foam stood for 90 min, while at acidic (pH 2) and alkaline (pH 8 and 10) the foam stability was increased.

Ulloa *et al.* (2010). A study was carry out the Physicochemical and functional properties of a protein isolate produced from safflower (*Carthamus tinctorius* L.) meal by ultrafiltration the effect of pH on the foaming properties of safflower protein isolate obtained by ultrafiltration. The maximum foaming capacity (126%) and foam stability (45.5%) were observed at pH 2 and 4 respectively, while the minimum foaming capacity (80%) and foam stability (9.7%) occurred at pH 6–8 and 6 respectively.

Khalid *et al.* (2003). This experiment was collected the Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration they resulted The foam capacity (FC) of sesame protein isolate was pH-dependent and was found to be lowest at pH 5 (2%). The lowest FC was attributed to the protein behaviour at its isoelectric point. Beyond pH 5, FC significantly increased, especially at pH 9 and 10. The higher FC at the above two pHs was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein.

Coffmann and Garciaj (1977). This data was performed by Functional properties and amino acid content of a protein isolate from mung bean flour so the Whipping of the mungbean protein isolate was carried out at room temperature, pH 7.0, and 8%protein concentration (w/v).

Ocloo *et al.* (2010). This experiment was conducted by Ghana its Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds this study was resulted The foam capacity of the jack fruit seed flour is 25.34 %.

2.4.5 Solubility

Nassar (2008). This experiment was conducted the Chemical Composition and Functional Properties of Prickly Pear (*Opuntiaficus indica*) Seeds Flour and Protein

Concentrate. The nitrogen solubility as a function of pH is show us. The data show three regions of nitrogen solubility, at acidic pH, near to the isoelectric point and at alkaline pH. The minimum nitrogen solubility was observed at pH 4.5 which was 16 % for PPS flour compared to 15% for protein concentrate, indicating the isoelectric point of the protein; in the acid media (pH1). 73% of the protein was soluble compared to 79% for protein concentrate. At pH 8, 51 and 56% of protein was soluble, while 85 and 92% was soluble at pH 11.

Ulloa *et al.* (2010). A study was carry out the Physicochemical and functional properties of a protein isolate produced from safflower (*Carthamus tinctorius* L.) meal by ultrafiltration so The nitrogen solubility of safflower protein isolate as a function of pH The data show three regions of nitrogen solubility: at acidic pH, near the isoelectric point and at alkaline PH The minimum nitrogen solubility was observed at pH 4 (7.3%), indicating the isoelectric point of the protein; at pH 2 and 9, 65.1 and 72.1% of the protein respectively was soluble. The nitrogen solubility at different pH values may serve as a useful indicator of the performance of protein isolates in food systems in addition to the extent of protein denaturation as a result of heat or chemical treatment. Most plant proteins have isoelectric pH values between 4 and 5. At the isoelectric point, there is no net charge on the protein; as a result, there are no repulsive interactions or protein–protein interactions disfavouring solubility.

Khalid *et al.* (2003). This experiment was performed the the Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration the result shown us the variations in nitrogen solubility at different pH levels of a total protein isolate (TPI). The minimum nitrogen solubility of TPI was 12% at pH 5 and also observed at pH 4 and 6. On either side of pH 4 and 6, there was a sharp increase in the solubility for the total protein isolate. At pH 3, about 90% of the nitrogen was soluble, and about 72% of the nitrogen was soluble at pH 10.

2.4.6 Gelation

Abbey and Ibeh (1988). A study was collected the functional Properties of Raw and Heat Processed Cowpea (*Yignaun guicukta*, Walp) Flour and the result Least gelation concentrations for the raw and heat processed cowpea seed flour were found to be 16% and 18% (w/v), respectively.

Coffmann and Garciaj (1977). This experiment was conduct the Functional properties and amino acid content of a protein isolate from mung bean flour they resulted Protein concentration of 10, 12 and 14% consistently gelled upon application of heat. Gels of 10 and 11% protein were softer than gels of 12-14% protein which were very stiff. Samples of 8 and 9% protein were very viscous. In fact, a coagulum was formed but it was not strong enough to prevent gel disruption and slippage when the test tube was inverted.

Samanta and Laskar (2010). A study was collected the Functional Properties of *Erythrina variegata* Linn. Seed Protein Isolate Gelation properties of the protein isolate solution were investigated and the lowest gelation concentration was found to be 10%.

CHAPTER III

MATERIAL AND METHODS

3.1 Materials

3.1.1 Sample collection

Ripen mango (*Mangifera indica* L.), Jackfruit (*Artocarpus heterophyllus* L.) and litchi fruits were collected from the local market of Dinajpur and seeds were collected by manually and kept at 4˚C until uses.

3.1.2 Chemicals and reagents

Hydrochloric acid, Sodium hydroxide, Boric acid, Copper sulphate, sulfuric acid, Fehling solution, Potassium sulphate, petroleum benzene, methyl red, Bromocresol green, etc. were purchased from Mark, Germany. All the chemicals and regents used in this study were of analytical grade.

3.2 Methods

3.2.1 Preparation of seeds flour

The washed seeds were sliced into small pieces (2-2.5 mm thickness) with knife or hammer. Then the seed was dried at a cabinet drier (model- 136-12, Seoul, Korea) at 60±10˚C for 24 hour followed by grinding into flour by using a grinder (Japan CM/L-7360065). The flour was sieved through a sieve (42 mesh size) and packed in a plastic bag. The obtained flour was sealed and stored in a refrigerator at -18˚C until further use.

3.2.2 Preparation of protein isolates

Extraction of proteins and preparation were taken as Sample of (50g) was added to distilled water at a ratio of 1:5 (w/v). The mixture was stirred with a magnetic stirrer for10 min and the pH of the solution was then adjusted to 9.0 using 1 MHCl or NaOH and stirring continued for another 30 min at room temperature. Each extract was separated by centrifugation at 3000 x g for RCF 30min. the supernatant was discarded and residues were re-extracted two more times with the same solvent under similar conditions. The extracts were combined and proteins precipitated by adjusting the pH to 4.5 with 1 MHCl, followed by separation by centrifugation at 3000 x g for 30 min. The

precipitate was re-dispersed in 100 ml distilled water at pH 9.0 and re-precipitated at pH4.5. After separation of proteins by centrifugation at 3000xg for 30 min, the precipitate was washed twice with distilled water (1:2: sample: water). The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1M NaOH prior to drying. The protein isolates was dried in oven dryer at 55c˚ temperature in 8h and stored in air-tight plastic-bag containers at freeze temperature for further analyses.

3.3 Characterization of protein

3.3.1 Determination of crude protein content

AOAC method 7.056 (2000) was used with some modification to determine the protein content of the seed powder. Usually three stages are used to determine protein content. These stages are given below:

A. Digestion

   Seed powder (0.1g), $CuSO_4$ (0.1g), K_2SO_4 (1g) were taken into a volumetric flask. Then 10ml of H_2SO_4 (conc.) was added. After that the volumetric flask was heated at 300°C for 1hrs and cooled for 20 minute at room temperature.

B. Distillation

After digestion 300 ml of distilled' water and 125 ml of 40% NaOH were added to the volumetric flask. 25 ml of 4% boric acid solution and 2-3 drops mixed indicator were taken in a conical flask. The volumetric flask was connected with one end of the condenser and the conical flask was connected with other end. The volumetric flask was heated continuously until the conical flask was filled to 150 ml.

C. Titration

   The conical flask was disconnected and was taken for titration. Titrated against 0.2 N of H_2SO_4 solutions. The end point was indicated by orange color.

Calculation

I) Calculation for N_2 content: % of N_2 = Burette reading x Normality of H_2SO_4 X ml equivalent of N_2

Here,

Normality of H_2SO_4 = 0.2

Ml equivalent of N_2 = 1.4

II) Calculation for protein content:

$$\% \text{ Protein} = \% \text{ of } N_2 \times \text{Proteir. Factor}$$

Here,

Protein factor = 5.5

3.3.2 Yield

Yield was calculated by following formula

$$\% \text{ yield} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

3.3.3 Determination of moisture Content

AOAC method 7.045 (2000) was used to determine the moisture content of jackfruit seed powder. Jackfruit seed powder (1g) was taken in a clean, dry and pre-weighted crucible. Then the powder was transferred to oven and dried at 105°C for 16 hours. After that it was cooled at desiccator and weighed. Again it was transferred to oven and dried until a constant weight was obtained. Finally, it was cooled and weighed.

Moisture Content was calculated by following formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1= weight of sample with crucible

W_2 = weight of dried sample with crucible w = weight of sample

3.3.4 Determination of ash content

AOAC method 14.006 (1975) was used to determine the total ash content. Jackfruit seed Powder (1g) was weighed and transferred into a clean, dry and pre-weighted crucible.

Then the crucible was kept into muffle furnace at 550°C for 6 hours. It was cooled at desiccator and weighed.

The ash content was calculated by the following:

$$\%\text{Ash} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1 = weight of ash with crucible w_2 = weight of empty crucible w w= weight of sample

3.3.5 Determination of crude fat

AOAC method 7.045(2000) was used with some modification to determine the fat content of the seed powder. Jackfruit seed powder (1g) was taken into the thimble. The thimble was attached to the Soxhlet apparatus which was attached with a saand bottom flask containing 200 ml ether. The fat was extracted for 8 hours. After that ether was evaporated at 80°C until the flask completely dried.

Fat content was calculated by following formula:

$$\%\text{fat} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W_1= weight of evaporated flask with sample W_2= weight of empty flask

W = weight of sample

3.4 Functional properties of protein

3.4.1 Bulk density

Monteiro and Prakash (1994) is a method we using determination of bulk density of protein extraction of plant by-product. A calibrated plastic centrifuge tube was weighed (W_1), protein samples were filled to 25 ml and the tubes were tapped to eliminate the

spaces between the particles, the volume was taken as the volume of the sample. The tube was weighed again (W_2). From the difference in weight, the bulk density of the protein samples was calculated and expressed as grams per milliliter (g/ml).

3.4.2 Water absorption capacity (WAC)

Rodriguez-Ambriz *et al.* (2005) this method we using the determination of water absorption capacity. 100 mg of each protein samples was mixed with 1000 ml of distilled water using a stirrer. The protein suspension was then centrifuged at 1800 x g for 20 min at 22 ℃. The supernatant was decanted, and the tube was drained at 45° angle for 10 min. Water absorption capacity was calculated by dividing the volume of water absorbed by the weight of the protein sample.

3.4.3 Oil absorption capacity (OAC)

Lin and Zayas (1987) by this method we using determination of fat absorption capacity; 100 mg of protein sample was vortex-mixed with 1000 ml of soybean oil for 30 s. The emulsion was incubated at room temperature (about 20 °C) for 30 min and then centrifuged at 13,600g for 10 min at 25 °C. The supernatant was decanted and drained at a 45° angle for 20 min. The volume of oil absorbed was divided with the weight of the protein sample, to obtain the fat or oil absorption capacity of the sample.

3.4.4 Foaming properties

Foaming capacity and stability of the protein extraction from different by-product were determined according to the method described by Sze-Tao and Sathe (2000). Two hundred and fifty milligrams of each protein sample were mixed with 250 ml of distilled water, and the pH was adjusted to 2, 4, 6, 8, and 10. This protein solution was whipped for 3 min in a stainless GS Blender (model 38 BL45 by Dynamic Corporation of America). The whipped protein solution was then poured into a 100 ml graduated cylinder. The total sample volume was taken at 0 min for foam capacity and up to 60 min for foam stability. Foam capacity and foam stability were then calculated:

$$\% \text{ Foam capacity (FC)} = \frac{(\text{Volume after whipping} - \text{volume before whipping}) \text{ ml}}{(\text{Volume before whipping}) \text{ ml}}$$

$$\text{Foam stability (FS)} = \frac{(\text{Volume after whipping} - \text{volume before whipping}) \text{ ml}}{(\text{Volume before whipping}) \text{ ml}}$$

3.4.5 Gelation

Gelation of the protein extraction from different plant by-product were determined using the method described by Abbey and Ibeh (1988); cashew protein samples were mixed with 5 ml of dis- tilled water in a centrifuge tube to obtain 2%, 8%, 16%, and 20% w/v concentrations. The centrifuge tube was heated for 1 h in a boiling water bath, cooled rapidly under running tap water and further cooled for 2 h in a refrigerator at 4 °C. The least gelation concentration was regarded as the concentration at which the sample from the inverted tube did not fall or slip.

3.4.6 Protein solubility in water

This was determined by the method described by Klompong *et al.* (2007), 200 mg of each protein sample was dispersed in 20 ml deionised water, and the pH of the solution was adjusted to 2, 4, 6, 8, 10, and 12 with 1 or 0.1 N HCl and 1 or 0.1 N NaOH. The mixture was stirred at room temperature (about 20 °C) for 30 min, using magnetic stirrer, and then centrifuged at 7500 x g for 15 min. The protein content in the supernatant was determined using the kjeldal method. Protein solubility was then calculated:

$$\text{Solubility (\%)} = \frac{\text{Protein content of supernatant}}{\text{Total protein content of the sample}} \times 100$$

3.5 Data analysis

Data collected from all experiments were in triplicate, and subjected to data analysis of Excel.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Proximate analysis

The proximate compositions of three different fruit seeds are shows in figure-2. The moisture content of all seed flours were varied between 3.68% to 16.60%. Highest moisture content was (16.60%) found in litchi seed whereas lowest moisture content was (3.68%) found in mango seed. These results were higher with the findings of Ocloo *et al.* (2010) who reported that the moisture content of jackfruit seed was 6.09%, also these value were consistent with Tulyathan and Jaiboon (2002) who resulted that the moisture content of jackfruit seed was 8.57%, This report has resulted the chemical composition of A. trifoliata var. australis seed that showed moisture content of protein isolation (13.01%) Y. Du *et al.* (2012), this finding is also similar with above result. The moisture content of mango and jackfruit seed being 15.93 and 16.60% this result therefore shows that MSP, JSP Seed has high moisture content hence cannot be preserved long time, this value is high when compared to 5.5 and 5.1% for cashew nut fetuge *et al.* (1974) and African oil been Osagie *et al.* (1986). Where the moisture content of total protein isolation of erythrina variegate seed was found (9.16%) Samanta and Laskar (2010).

The ash content of three different by-product of fruits MSP, JSP AND LSP protein isolate shown as 1.36%, 1.23% and 2.07%, respectively. Furthermore, litchi seed protein isolate contained little bit high amount of ash then the other seed. This report showed ash content of jackfruit seed flour was 3.97% VannaTulyathan *et al.* (2002), this report shown us higher, so different can be contributed by variety difference, maturate of the seed and environmental conditions. These effects were already reported by Rahman *et al.* (1999). Were ocloo *et al.* (2010) reported that ash content of flour was 2.70%. These results also lower then that found by Morton, (1987) who recorded ash content of jackfruit seed was 2.76- 3.31%. The variation in ash content in different varieties jackfruit seed flour might be due to the locality (Ocloo *et al.,* 2010). Where khalid *et al.* 2012) reported cowpea seed flour of Whole Cowpea Flour (WCF) AND dehulled defatted cowpea flour (DDCF) were resulted that 3.77 to 3.87% of ash, this finding was obtained and comparable with this data that reported by Abdalla *et al.* (2001) and also with Sosulki *et al.* (1987).Where ash content of beach pea isolate was 5.99% ash that

was reported by U.D. Chavan *et al.* (2001), this comparable with data that shown in figure-2, while5.99% ash is obviously higher then which ranged 1.36 to 2.07% due to this currant data.

In this study, shown high variation of fat content among different three by-products of mango, jackfruit and litchi seed. The oil content in three different fruits seed (MSP, JSP AND LSP) are showing in Fig-2 are 14%, 2% and 1%, respectively. As we seen the figure the highest fat content is MSP compared others and its contain 14% oil. This result was higher than compared to pearl millet (7.6%) and quinoa (6.3%), Oshodi *et al.*, (1999). The result of jackfruit and litchi seed (2.70 and 1%) are close to pigeon pea flour (1.80%, Okpala and Mommah, 2001) and wheat flour (3.10%, Akubor and Badifu, 2004). These value were similar with the finding reported by Ocloo *et al.* (2010) who found that fat content of flour was 1.27%. This value was similar finding reported by Chavan *et al.* (2001) who resulted that fat contain of beach pea protein isolation was 3.20% fat.

Figure-2 shown protein content of protein extraction by-product of three different fruits such a mango, jackfruit and litchi seed were 28.23%, 43.12% and 36.96%, respectively. Protein extraction from jackfruit seed had highest protein value (43.12%) as compared to mango (28.23%) and litchi protein isolation seed (36.96%). Significant difference was observed in protein content in all extraction protein samples. Where the protein isolation of beach pea was prepared using solvent extraction with NOAH and SHMP solution, who resulted the NOAH extracted beach pea protein isolate had 86.6% while SHMP extracted shown it contained 85.1% protein, Chavan *et al.* (2001), and also these result is higher than my own due to verity. These values were close to those reported by Samanta and Laskar (2010) who observed that protein content of erythrina variegate linn seed protein isolation was 71.38%. The difference in protein value might be due to differences in protein isolation procedure.

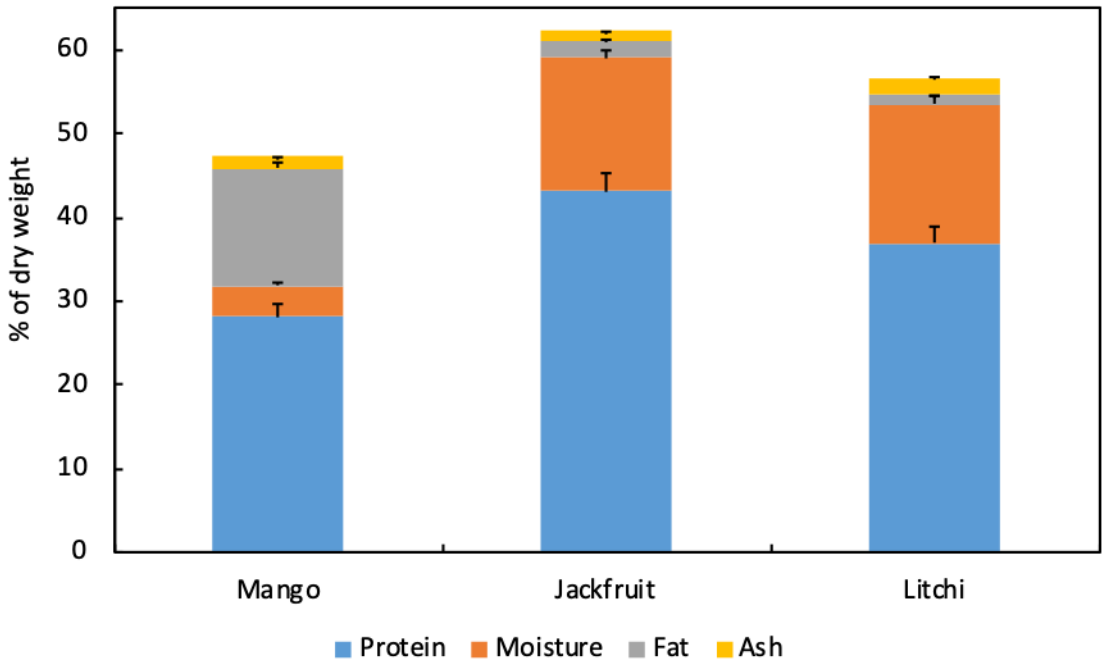

Figure 2: General compositions of mango, jackfruit and litchi seed protein extraction

4.2 Functional properties

4.2.1 Solubility

Solubility of a protein is one the critical functional attributes required for its use as a food ingredient, because solubility directly influence other functional properties such as gelation and foaming, therefore protein solubility a very important for functional properties, can also be significantly affected by ph. high solubility indicates or showing us quality of the protein (Kinsella and Melachouris, 1976).

Figure 3 shows the protein solubility of different isolated protein seeds from (MSP, JSP, LSP) at different pH levels between 2 to 12. The minimum solubility of mango, jackfruit, litchi protein seeds differently were found to be 10.91%,7.14% and 18.68%, respectively at pH 4, while the solubility were showed in similar results at pH 2. The solubility of defatted three different seed protein increased from pH 6 to pH 12, and the maximum solubility was obtained 27.27%, 21.42% and 20.75%, respectively at pH 12. The effects of pH on the solubility of this three different fruits protein are shown in Fig.2. The highest solubility was mango protein seed and shown (27.27%) at pH 12 while jackfruit protein seed decreased to the minimum level of 7% at pH 4.0. The solubility thereafter increased from pH 6 to pH 12 to reach the maximum solubility of the pH 12. Fig. 2 shows the effects of pH on the solubility of different fruit protein (MSP, JSP AND LSP), by-product protein isolate was highly soluble at both acidic and basic ph. The minimum solubility of 7 to 10% was obtained at pH 2 and 4, while the maximum solubility 27 to 21% was obtained at pH 10 and 12. The solubility of MSP, JSP, and LSP in water at different pH showed which look like U-shaped pattern, which are closed off and similar to many such profiles reported for peanut proteins (Monteiro and Prakash, 1994). Fig.2 showed the differences in protein solubility at different pH levels of a total protein isolate. The minimum nitrogen solubility of TPI was jackfruit 7% at pH 4 and also observed at pH 2 similar. The either side of pH 4 and 6, there was a sharp increase in the solubility for the total protein isolate. Total protein isolate studied showed good solubility in both acid and alkaline pH regions, which is an important characteristic for food formulations (Idouraine, Yensen, and Weber, 1991). Prakash and Narasinga Rao (1986) reported closely observations. This data showed three regions of nitrogen solubility: at acidic pH, close the isoelectric point and at alkaline ph. The minimum nitrogen solubility was noticed at pH 4 (7.3%), indicated the isoelectric point of the

protein, at pH 2 and 9, (65.1 and 72.1%) of the protein respectively was soluble. The protein solubility at different pH values may serve as a useful indicator of the performance of protein isolates in food systems in addition to the extent of protein denaturation as a result of heat or chemical treatment. Most plant proteins have isoelectric pH values between 4 and 5. At the isoelectric point, there is no net charge on the protein; as a result, there are no repulsive interactions or protein–protein interactions disfavouring solubility (Ulloa *et al.*, 2011). This report were closely similar to my own that resulted the protein solubility of three samples showed us-shaped, decreased at first and then raised up steadily from ph. 5 and 10. The minimum solubility of API (2.10 %) albumin (9.49%) and gluten (3.02%) was noticed at ph. 4 and 5 (Du *et al.*, 2012).

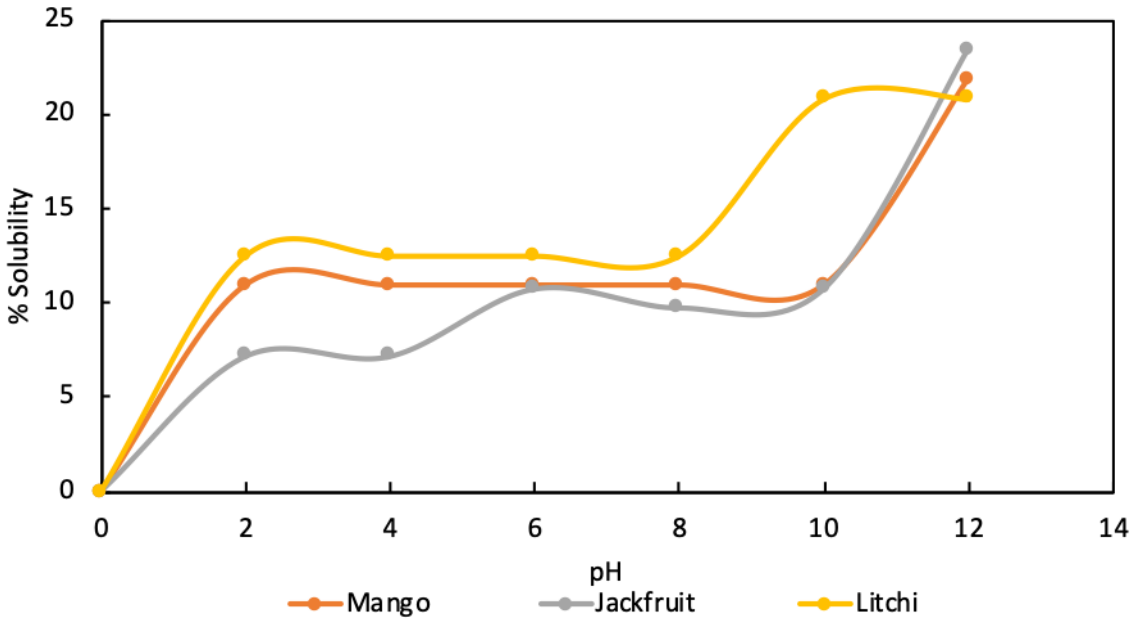

Figure 3: Effects of pH on water solubility of mango, jackfruit and litchi seed protein extraction

4.2.2 Foaming properties

4.2.2.1 Foaming capacity and foaming stability

The foaming formation is important in food applications such as beverages, mousses, meringue cakes and whipped toppings (Boye *et al.,* 2010). The foaming capacity (FC) of thee different by-product such mango, jackfruit and litchi seed protein isolate shown Figure 4, were Ph dependent and found to be lowest at Ph 10 (0.38 %,4.23% and 0.16% respectively), the lowest FC was attributed to the protein behaviour at isoelectric points. The highest FC was so two sample of the mango and litchi was Ph6 where at higher FC of jackfruit wasPH2 (1.95%, 1.19% and 17.18% respectively). The higher FC at the above three phs was due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air- water interface to encapsulate air particles and then enhance the foam formation (Aluko and Yada, 1995). Figure 4 shows the foaming capacity of mango, jackfruit and litchi seed protein isolate was varied between 17.18% to 0.16%. these results also were lower than that reported by Ogunwolu *et al* (2009) who recorded the foaming capacity of cashew nut protein concentration (CNPC) was 45%, were cashew nut protein isolation was 40%.which is higher than values reported for pearl millet flour and quinoa flour (11.30 and 9 % respectively)

(Oshodi *et al.,* 1999). However, this value is comparable to values reported for African breadfruit kernel flour and wheat flour respectively (20 and 40 %) (Akubor and Badifu, 2004). Foam ability is reported to be related to the amount of solubilized protein (Narayana and Narasinga Rao, 1982; Lin *et al.,* 1974) and the amount of polar and non-polar lipids in a sample (Nwokolo, 1985). Therefore, to exhibit good foaming, a protein must be capable of migrating at the air–water interface, unfolding and rearranging at the interface (Halling, 1981). According to Damodaran (1997), the foam capacity and stability were enhanced by greater protein concentration, because this increases the viscosity and facilitates the formation of a multilayer, cohesive protein film at the interface. The foam stability (FS) of protein isolates in mango, jackfruit and litchi seed are summarized figure-5 and its shown us foam stability variance between 0 to 12.40 %, the highest foam stability was jackfruit in ph4 (12.40%) where mango and litchi have no foam stability. Foam stability is the volume of foam remaining after a specified time as a percentage of the initial foam volume. Were evaluation by Ogunwolu *et al.* (2009) who

resulted the defeated cashew nut powder, cashew nut protein powder and cashew nut protein isolate was (55%, 40% and 8% respectively), as we see the cashew nut protein isolate is low foam stability comparing to others. As the time of standing progressed, the foam volume was decreased. A similar trend was observed for cowpea (Aluko and Yada, 1995), soybean, and sunflower protein isolates (Lin, Humbert, and Sosulski *et al.*, 1987). Kinsella and Melachouris (1976) and Myers (1988) have suggested that, in foams, the ability to hold water in the protein film surrounding the air particle and presence of electrostatic repulsions are important for their stability.

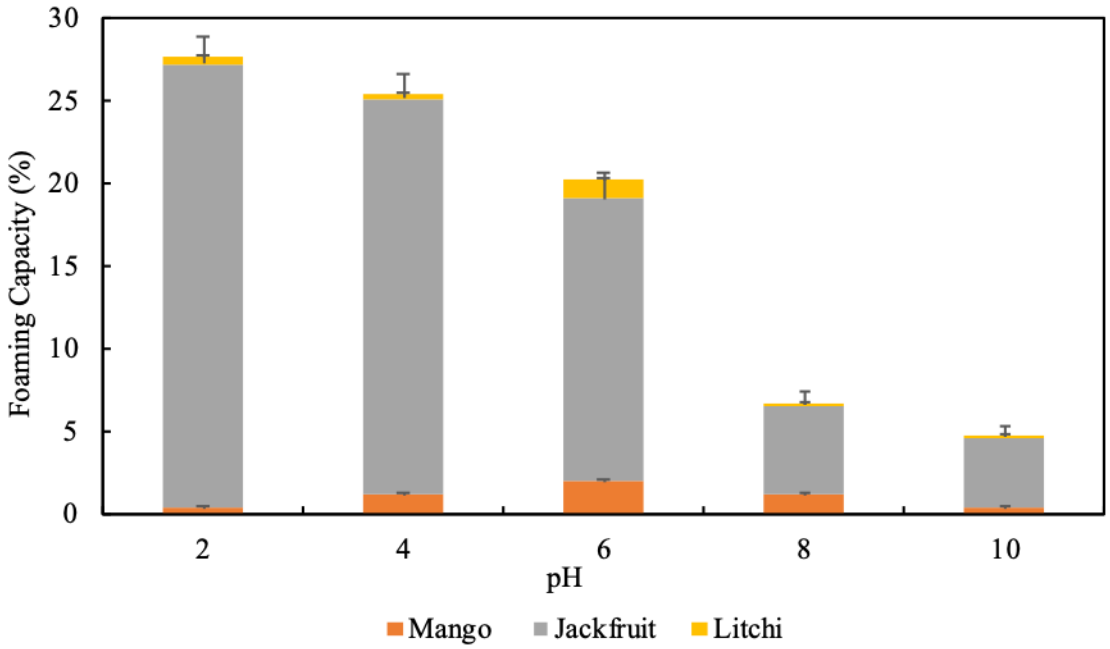

Figure 4: Effects of pH on foaming capacity of mango, jackfruit and litchi seed protein extraction

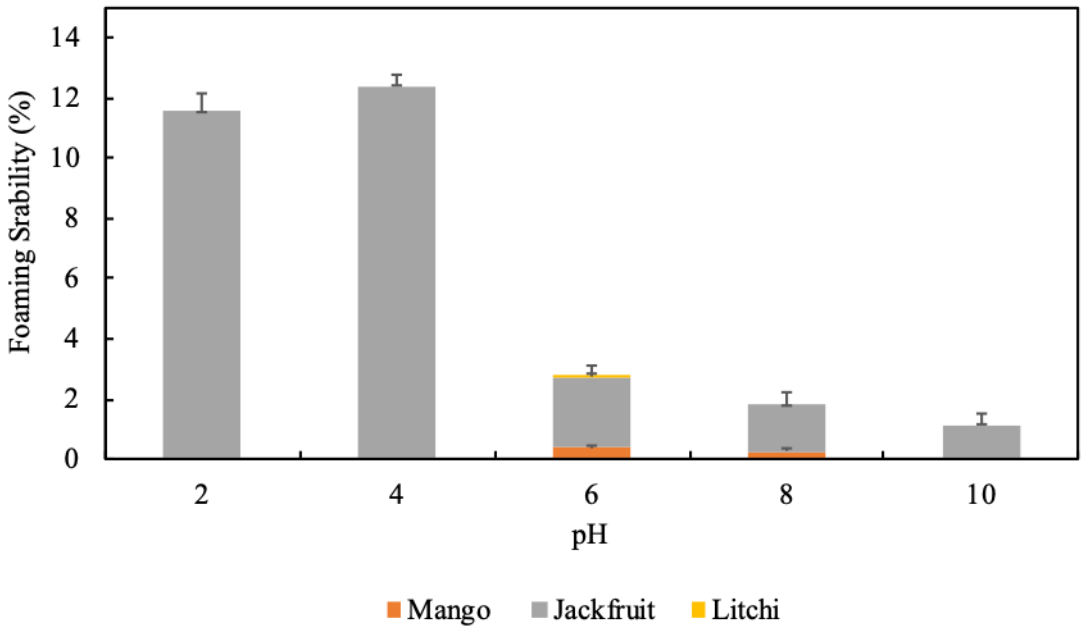

Figure 5: Effects of pH on foaming stability of mango, jackfruit and litchi seed protein extraction

4.2.3 Water and fat absorption capacity

The Table-1 shows the water absorption capacity (WAC) of three by-product plant seed (MSP, JSP AND LSP) was 1.48, 2.99 and 2.26 ml H_2O/g respectively, this result shown the mango protein extraction is poor water-binding capacity comparing to the jackfruit and litchi protein extraction. This value was higher than that found by Ogunwolu *et al.* (2009) who reported WAC of the defatted cashew nut powder (DCNP) 0.8 ml/g, and defeated peanut powder 1.45 ml/g, as reported by Monteiro and Prakash (1994). Where The WACs of PCs from some other legume seeds such as *Phaseolus mungo* L., *Phaseolus vulgaris* L., and sunflower seed were 5.90, 5.93, and 3.90 ml/g, respectively (Sathe, 1983; Sathe and Salunkhe, 1981; Sosulski and Fleming, 1977). Basically, the differences in WAC among these legume PCs might be attributed to the different protein conformations and the variations in the number and nature of the water-binding sites on protein molecules (Chou and Morr, 1979), also WAC of the protein is closely related with its amino acid profile, protein conformation, surface hydrophobicity, pH, and temperature (Barbut, 1999) as well as lipids and carbohydrate associated with the protein (Mao and Hua, 2012).

Fat absorption is an important property in food formulations because fat improves the flavour, texture and mouth feel of foods (Kinsella and Melachouris, 1976). Oil absorption capacity (OAC) of mango, jackfruit and litchi seed protein isolate was found to be 1.393 ml oil/g, 1.488 ml oil/g and 1.528 ml oil/g respectively, highest oil absorption is litchi protein isolate 1.52ml oil/g, comparing to the other samples. The oil absorption capacity of defatted peanut powder was 1.79 ml oil/g, reported by Beuchat *et al.* (1975), but the comparable with that of defatted Niger seed was 2.00 ml oil/g reported by Bhagya and Sastry (2003). Where thus value is similar then that evaluated by khalid *et al.* (2012), who reported the oil absorption capacity of Dehulled defatted cowpea flour (DDCF) was 1.04 ml oil/g while oil absorption capacity cowpea protein isolation (CPI) was resulted 1.93 ml oil/g. which was comparable to that reported for cowpea flour (Abu *et al.,* 2005).

4.2.4 Bulk density

Bulk density is depending upon the particle size of the sample and it is important for determining packaging requirements, material handling and application in wet processing in the food industry (Chandi and Sogi, 2007). The table-1 shown the value

obtained in three different samples mango, jackfruit and litchi seed protein isolate were (0.638 g/ml, 0.517g/ml and 0.63 g/ml respectively). while this finding similar with Dongyan *et al.* (2013) resulted by The bulk density of hot tomato seed (HTS), defeated hot break seed (DHTS) and defeated cold break tomato seed (DCTS) were found to (0.73 g/ml, 0.62 and 0.61 g/ml, respectively).Where Ogunwolu *et al.* (2009) reported that bulk density of defatted cashew nut powder (0.48 g/ml) was found to be higher than that of cashew nut protein concentrate (0.31 g/ml), which was in turn higher than that of cashew nut protein isolate (0.25 g/ml). Bulk density depends on the combined effects of interrelated factors such as the intensity of attractive inter-particle forces, particle size, and number of contact points (Peleg and Bagley, 1983). In some cases, e.g., in convalescent feeding, higher bulk density is desirable since it helps to reduce the paste thickness. However, high bulk density is disadvantageous for the formulation of weaning foods, where low bulk density is required (Chandi and Sogi, 2007).

4.2.5 Gelation capacity

Gelation is an aggregation of denatured molecules. Table-1, shown the least gelation capacity (LGC) of the different sample of mango, jackfruit and litchi seed protein isolate were (1 %, 6% and 2% respectively). This result noticed low gelation capacity comparing with Okezie and Bello (1988) who reported by soy bean powder gelation capacity (14%). Lqari *et al.* (2002) reported that LGC of lupin protein concentrate is 12%, while Schmidt (1981) reported 7.5% for wheat protein isolate. This report similar with Lqari *et al.* (2002) who resulted the least gelation concentrations of lupin protein isolate A and B were respectively, 12 and 10%. Chau and Cheung (1998) and Sathe *et al.* (1981) reported that the least gelation concentrations of the lupin seed flour and the protein concentrate were 14 and 8% (wlv), respectively. The MSP and LSP isolate in table-1 shown low protein gelation comparing to JSP. In this sense, the gelation is not only a function of protein quantity but seems also to be related to the type of protein as well as to non-protein components, as suggested by Sathe *et al.* (1981) and Tjahjadi *et al.* (1988).

Table 1: Functional Properties of mango seed protein (MSP), jackfruit seed protein (JSP) and litchi seed protein (LSP) at their natural pH.

Functional Property	Mango Seed	Jackfruit seed	Litchi Seed
Bulk density	0.608 ± 0.11	0.517 ± 0.09	0.636 ± 0.14
WAC (ml/g)	1.482 ± 0.23	2.998 ± 0.42	2.266 ± 0.31
FAC (ml/g)	6.831 ± 1.08	6.827 ± 1.22	6.531 ± 1.03
LGC (%)	1.00 ± 0.21	6.00 ± 0.32	2.00 ± 0.22

CHAPTER V

CONCLUSIONS

In this study, protein flour was prepared from fruit by-product of mango, jackfruit and litchi seed. The result showed that moisture content was higher in litchi seed protein extraction as compared to mango and jackfruit seed protein extraction, also litchi seed protein extraction had high ash content. Where mango seed protein extraction has high fat amount extraction. This experiment also showed that the protein solubility of litchi seed is higher while compared to mango and jackfruit seed. The protein extracted from jackfruit seed showed higher water absorption capacity. The fat absorption capacity of mango and jackfruit seed were almost similar but higher then litchi seed protein. The overall results showed that mango, jackfruit and litchi seed protein extraction might be used protein supplement.

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APPENDIX I: Experimental Photograph

Jackfruit seed

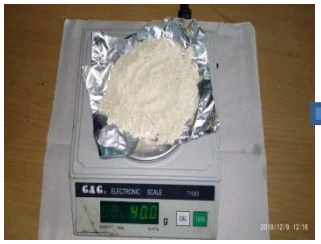
Litchi seed

Mango seed

Jackfruit seed powder

Litchi seed powder

Mango seed powder



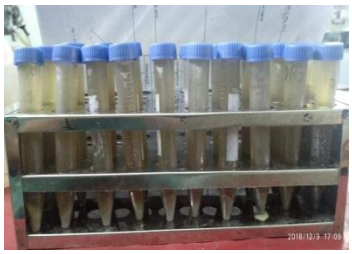
Measurement of sample

DW

Reagents of HCL, NoaH

Adjustment of PH.

Centrifuge



Test Tube with sample

Supernatant

Precipitated

Sediment protein

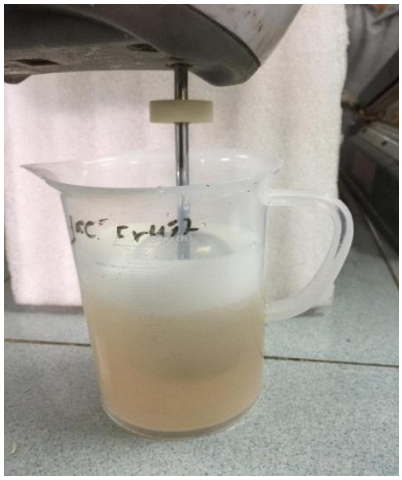
Oven dryer

Protein extraction

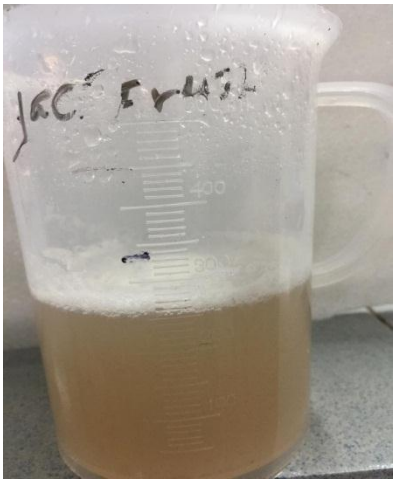
Digestion

kjeldahl apparatus.
(Distillation)

Titration



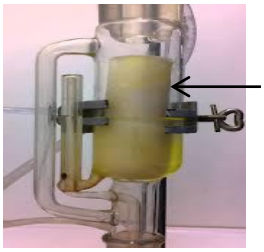
Foaming capacity



Foaming stability

Oven drying

Muffle furnace



Soxhlet apparatus