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Physicochemical and Functional Properties of Protein Isolate from Ackee (*Blighia sapida*) Seed

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Abstract: *Ackee seeds are a major waste of the ackee canning industry. The seeds bio-accumulate the toxin hypoglycin but are also a potential source of protein. The objective of this study was to produce a protein isolate from the ackee seed and determine the chemical and functional properties of the isolate. Proteins were extracted from the defatted ackee seed flour using sodium borate buffer (pH 10.0, 50 mM) and aqueous ethanol (75% v/v). The protein isolate was then dried under vacuum and milled into a powder. The ackee seed protein isolate contained low molecular weight proteins comprising principally glutamic acid, arginine, glycine and aspartic acid with hypoglycin content within regulatory limits. Ackee seed protein powder, in comparison to soy protein isolate, demonstrated high solubility, formed stable emulsions and demonstrated good foaming properties, particularly at acidic pH values, making it suitable for use in acidic foods such as fruit juices, beverages and yoghurts. The purity (g protein/100 g isolate) of the ackee seed protein isolate was found to be lower than that of the commercial soybean protein isolate while higher quantities of fat and ash were present in the soybean protein isolate. The water and oil absorption capacities (g/g isolate) of ackee seed protein were lower than those of the commercial soybean protein isolate. Hypoglycin content in the ackee seed protein isolate was determined to be below the commercial standard for ackee products.*

Keywords: *Ackee seed, Blighia sapida, protein, functional properties, solubility, foaming, emulsification*

1. Introduction

Proteins contribute significantly to the textural, sensory and organoleptic properties of foods through their functional roles of binding of water and fats, formation and stabilisation of emulsions, production of foams and formation of gels (Zayas, 1997; Culbertson, 2005; Chandra et al., 2015). Protein isolates (70-90% protein dry basis (db)) can provide valuable additional nutrition and functionality in foods (Ghribi et al., 2015). The functionality of protein isolates is influenced by the physical and chemical properties, their interactions, and extrinsic factors such as pH, temperature, and ionic strength (Zayas, 1997; Culbertson, 2005; Chandra et al., 2015). Protein isolates obtained from soybeans (soy protein isolates) are widely used to emulsify fats and bind water which allows the incorporation of oil into food products as well as fortify cereal meals that are often deficient in one or more essential amino acids (Sing et al., 2008). Increased focus is now being paid to the extraction and characterisation of protein isolates from novel protein sources such as unconventional oilseeds and legumes, leaves, potato, algae, yeast and bacteria (Brown, 2020). One such promising alternative protein source is the seed of the ackee fruit, which is a waste product of the ackee canning industry.

Ackee (*Blighia sapida*) was introduced to the West Indies in the late 18th century and is the national fruit of Jamaica. In the local cuisine, the arils of the mature fruit (see Figure 1(a)) are normally boiled and eaten with codfish (Rashford, 2001). Arils of the immature fruit are not consumed as they contain the toxic compound hypoglycin A (HGA) (Jackson-Malete et al., 2015). Commercially, mature arils are canned in brine and exported from Jamaica, Haiti and Belize to over 25 countries, including the USA, Canada and the UK (USFDA, 2020). In 2019, the total value of ackee exports from Jamaica was just over USD 20 million (STATIN, 2020).

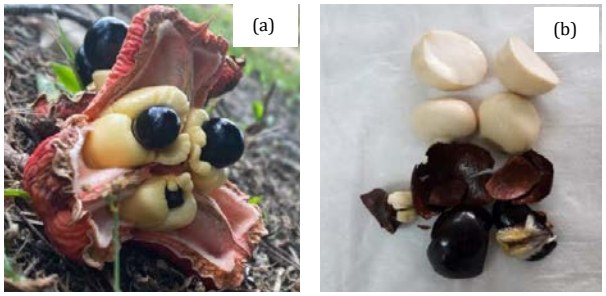

Figure 1: Split ackee fruit showing (a) arils and seeds, and (b) seed cotyledons separated from shells (dehulled seed)

Unlike the arils, the seeds of the ackee fruit are

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discarded in large quantities as a processing waste (Parkinson, 2007). Ackee seeds comprise a hard, protective outer shell (hull) that adheres to the cream cotyledons (Fig. 1B). Ackee seeds remain toxic even when fully mature as they contain hypoglycin A (HGA) and hypoglycin B (HGB) (Bowen-Forbes and Minott, 2011; Nwozo, 2014). Studies revealed that starch (44%), lipids (22%) and protein (21%) are the principal dry matter components of the dehulled seed (Djenontin et al., 2009; Esuoso and Odetokun, 1995). The oil of ackee seeds consists predominately of oleic and linolenic acids, and saponification values indicate that it may be suitable for use in making soaps and shampoos (Esuoso and Odetokun, 1995).

Work on defatted, dehulled ackee seed flour revealed that the isolated starch was of the C-type, had a high amylose content and comprised of small granules suggesting that the starch could be used in the production of retrograded resistant starch, bioplastics, fat replacers and cosmetic/dusting powders (Falloon et al., 2020). Akintayo et al. (2002) found the dehulled, full-fat ackee seed flour to have higher water absorption and oil absorption capacities but poor foaming properties in comparison with soybean and wheat flours. The authors further reported that protein solubility and emulsion capacity of the ackee seed flour was moderate when compared with other flours. The ackee seed flour was reported to have excellent gelling properties in that relatively small amounts (4% w/v) formed stable gels. Protein studies on the defatted flour of dehulled ackee seeds showed that glutamic and aspartic acids are the major amino acids with moderate quantities of the essential amino acids lysine, leucine, isoleucine, phenylalanine, and valine (Djenontin et al., 2009; Abiodun et al., 2015). No studies have, however, been reported on the isolation and functional properties of protein isolate from ackee seeds.

The objective of this study was to produce a protein isolate from ackee seeds and investigate the physicochemical and functional properties of the isolate. It is anticipated that the pure ackee seed protein isolate would have superior functional properties compared with that previously reported for the ackee seed flour, which could contain other components such as fats, fibres and carbohydrates.

2. Methods

2.1 Sample Collection and Preparation

Ackee seeds were collected from a processor in Jamaica. The seeds were washed, frozen, and shells removed using a hammer. The dehulled cotyledons were dried at 60ºC for 24 h in a forced draft convection oven (Environette, Lab Line Instruments Inc., Illinois). Dried seeds were milled (Model 4-E Quaker City Mill, The Straub Company, Philadelphia) to pass through a 0.5 mm pore size sieve and stored in re-sealable LDPE bags at 4ºC. The flour was defatted by Soxhlet extraction using petroleum ether (b.p. 60-80ºC).

2.2 Protein Isolation

Protein extraction was based on the method described by Wallace et al. (1990). Defatted ackee seed flour was suspended in a borate buffer ($Na_2[B_4O_5(OH)_4].10H_2O$/NaOH, pH 10.0, 50 mM), using a flour/buffer ratio of 1:5 w/v. The mixture was centrifuged (6400g, 10 mins, 25ºC) using a Sorvall RC 6 Plus, Thermo Fisher Scientific, Asheville, NC. The residue was re-extracted with the borate buffer, and the supernatants combined and chilled to 4ºC.

Protein was precipitated according to the method of Bollag et al. (1996). The chilled protein solution was placed in an ice bath and its pH adjusted to 4.0 using HCl (1 M). Reagent grade ethanol, chilled to -18ºC, was added to produce a 3:1 ethanol to protein solution volume ratio. This solution was stored at -18ºC for 1 h for protein precipitation to equilibrate. The suspension was centrifuged (18000g, 10 min, 0ºC). The crude precipitate collected was purified by re-dissolving in borate buffer and re-precipitated. The purified precipitate was dried in a vacuum oven (30-33ºC, 48 h, 20-24 Inch Hg), milled using a mortar and pestle, and stored at 4ºC in re-sealable air-tight LDPE bags.

The commercial soybean protein isolate was obtained from MP Biomedicals LLC, Solon, OH, USA.

2.3 Chemical Composition of Protein Isolates

The purity of the ackee seed and commercial soybean protein isolate was determined by estimating the protein content using the Kjeldahl (AOAC (2012) Official Method 2001.11) and Biuret (Gornall et al., 1949; Klompong et al. 2007) methods. Moisture was determined using Convection Oven Drying at 135^0ºC for 2 h (AOAC (2012) Official Method 930.15). Residual starch was determined using enzymatic hydrolysis (AOAC (2012) Official Method 996.11). The ash and crude fat contents of the isolates were determined according to the AOAC (2012) Official Method 942.05 (dry ashing) and AOAC (2012) Official Method 2003.03 (Randall Extraction-Submersion Method), using petroleum ether instead of diethyl ether. The analyses were conducted in triplicates and reported as g/100 g (dry weight basis).

The HGA and HGB content of the ackee seed protein isolate were determined in triplicates by reverse phase HPLC using the method described by Sarwar and Botting (1994) and expressed in terms of total HGA equivalent according to equation (1).

$$HGA\ Equivalent = [(M_w\ HGA/M_w\ HGB) \times Conc.of\ HGB] + Conc.of\ HGA \quad (1)$$

Where, M_w = molecular weight; Conc. = concentration.

2.4 Amino Acid Composition

The amino acid composition of the protein isolates was determined in triplicates (g/100 g protein isolate) using reverse phase HPLC according to the method described by Gonzalez-Castro et al. (1997).

2.5 Water Absorption Capacity (WAC)

Water absorption capacity of the protein isolate was determined according to AACC (1999) Method 56-30. Water absorption (g H_2O/g protein isolate) was

calculated, using three replicates, as stated in Equation (2).

$$WAC = (B - A) \div A \quad (2)$$

Where, A = Initial weight of protein isolate (g);
B = Weight of hydrated protein (g).

2.6 Oil Absorption Capacity (OAC)

Oil absorption capacity (OAC) was determined according to the method described by Wani et al. (2011). Corn oil (5 ml, supplied by ConAgra Foods, Omaha, NE 68103) was added to 0.5 g of protein isolate. Mixtures were vortexed (VWR Mini Vortexer MV1, Wilmington, NC) for 1 minute at 2000 rpm and stand for a further 5 minutes. This process was repeated five additional times. The mixture was centrifuged at 4000g for 15 minutes, and decanted. The tubes inverted on paper towels for 10 minutes. OAC (g oil/100 g protein isolate) was calculated, in triplicates, according to Equation (3).

$$OAC = (D - C) \div C \quad (3)$$

Where, C = Initial weight of protein isolate (g);
D = Weight of oil-saturated protein (g)

2.7 Solubility in Britton-Robinson Universal Buffer

The solubility of the seed protein isolates was determined according to the procedure of Klompong et al. 2007. Ackee seed protein isolate (ca. 50 mg) was added to 5 ml of Britton-Robinson Universal Buffer (Mongay and Cerda, 1974) at varying pH (2.0, 3.4, 4.5, 6.0, 7.5, 9.0 and 10.5) and ionic strength (0.1 M, 0.5 M and 1.0 M) combinations. The Britton-Robinson Universal Buffer consisted of acetic acid (0.04 M), phosphoric acid (0.04 M) and boric acid (0.04 M) (Reynolds III et al., 2013). The buffer was adjusted to the required pH and ionic strength combinations by using an appropriate volume of 0.2 M NaOH and an appropriate mass of NaCl (Mongay and Cerda 1974).

The mixtures were stirred for 30 minutes on a magnetic stirring plate, and centrifuged (7650g, 10 min). Solubilised protein in the supernatant was quantified using the Biuret Method, using Bovine Serum Albumin as the calibration standard. The total amount of protein in the protein isolates was determined by dissolving the isolates in 0.1 N NaOH (Aluko and Yada, 1995; Adebiyi and Aluko, 2011) prior to quantification.

Protein solubility (g protein/100 g protein) was calculated in triplicates according to Equation (4).

$$Protein\ Solubility\ (\%) = [F \div (E \times G)] \times 100 \quad (4)$$

Where, E = Weight of protein isolate (mg); F = Amount of solubilised protein (mg); G = Protein content (%) of isolate/100

2.8 Emulsion Capacity and Emulsion Stability

Emulsion capacity was determined using the method described by Chel-Guerrero et al. (2002). Protein isolate (0.52 g) was added to 35 ml distilled water. The mixture was stirred for 30 minutes on a magnetic stirring plate to solubilise the proteins. Corn oil (35 ml) was added to the protein dispersion, and the mixture was blended (Waring Commercial, Torrington, CT 06790) for 1 minute at low speed. Aliquots (20 ml) of the emulsified mixture were centrifuged at 1200 g for 5 minutes. The emulsion capacity was determined in triplicates according to Equation (5).

$$Emulsion\ capacity\ (\%) = (H \div I) \times 100 \quad (5)$$

Where, H = Height of emulsified layer (cm);
I = Total height of mixture (cm)

The experiment was repeated using 35 ml of Britton-Robinson Buffer, at varying pH/ionic strength combinations (see Section 2.7).

Emulsion stability was based on the method of Chel-Guerrero et al. (2002). The tubes containing the mixtures, from which emulsion capacity was determined, were immersed in a water bath at 80°C for 30 minutes. The mixtures were allowed to cool to ambient laboratory temperature (24°C) and centrifuged at 1200g for 5 minutes. The emulsion stability was done using three replicates and calculated according to Equation (6).

$$Emulsion\ stability\ (\%) = [((J \div K) \times 100) \div Emulsion\ Capacity] \times 100 \quad (6)$$

Where, J = Height of emulsified layer after heating (cm);
K = Total height of mixture after heating (cm).

2.9 Foaming Capacity and Foaming Stability

The method described by Chavan et al. (2001) was used to estimate foaming capacity. A 1% protein dispersion was prepared in distilled water and blended at low speed (Waring Commercial) for 1 minute to produce foams. The foam volume was immediately recorded using a measuring cylinder. The foaming capacity (three replicates) was calculated as percentage overrun according to Equation (7). The experiment was repeated using Britton-Robinson Buffer (50 ml) as stated in Section 2.7.

$$Foaming\ capacity\ (\%) = [(L - M) \div M] \times 100 \quad (7)$$

Where, L = final foam volume immediately after blending;
M = initial volume of mixture (50 ml)

Foam stability was determined by monitoring the decrease in foam volume after 1, 5, 10, 30, 60, 90 and 120 minutes after blending. Equation (8) was used to calculate foam stability (Deng et al., 2011; Timilsena et al., 2016; Pedroche et al., 2004).

$$Foam\ stability\ (\%) = (V_t \div V_0) \times 100 \quad (8)$$

Where V_t = foam volume at time (t = 2 h);
V_0 = Initial foam volume at zero time.

For the commercial soybean protein isolate, foam stability was recorded after 1 h, since in some cases, the foams completely dissipated within 90 minutes.

2.10 Protein Molecular Weights

Proteins were extracted from ackee seed protein isolate and commercial soybean protein isolate to produce a final protein concentration of 4-5 mg/ml. The extracting buffer (pH 8.0) consisted of 1% SDS, 0.05 M Tris base (tris (hydroxymethyl) aminomethane) and 1 mM dithiothreitol (DTT) (Sefton, 1997).

The molecular weights of the solubilised proteins

were estimated using one dimension sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). The resolving gel was a 4-20% acrylamide gradient pre-cast gel (Bio Rad Laboratories, Hercules, CA). Electrophoresis was carried out using a Bio-Rad Mini-Protean II apparatus at 150 V for 80 minutes. The molecular weights of the proteins were determined using pre-stained protein standards (6-199 kDa, Bio Rad Laboratories).

2.11 Statistical Analyses

Statistical analyses (Mean, Standard Deviation, ANOVA and Multi-Variable Interactions) were performed using IBM SPSS Statistics Version 21 (2015) (IBM Corporation, Armonk, NY) and Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA). All analyses were done in triplicate.

3. Results and Discussion

3.1 Moisture and Protein Contents of Ackee Seed and Defatted Flour

The moisture content of dehulled ackee seeds averaged 50.46 % (wb) or 1.23 g H_2O/g dm (see Table 1). The protein content of whole seeds, dehulled seeds and defatted flour ranged from 4.32 to 13.65% (wb). Protein yield from the defatted flour and whole seeds averaged 5.54 ± 0.42% and 1.70 ± 0.13%, respectively.

Table 1. Protein and Moisture Contents of Ackee Seed and Defatted Flour

Component	Ackee Sample		
	Whole Seeds	Dehulled Seeds	Defatted flour
Moisture % wb	55.24 ± 0.89^{c}	50.46 ± 1.11^{b}	5.99 ± 0.55^{a}
Protein % wb	4.32 ± 0.04^{a}	6.38 ± 0.07^{b}	13.65 ± 0.14^{c}

Values represent mean ± standard deviation, N = 3;
[a-c] Values sharing at least one letter in a row are not significantly different (95% CI)

The protein and moisture contents of the ackee seed defatted flour have not been previously reported. Other studies have however published data on the composition of the full-fat flour. Protein contents of 21.5% and 23.8% of the dehulled full-fat flour have been reported by Akintayo et al. (2002) and Esuoso and Odetokun (1995) respectively. The said flours were reported to have moisture contents of 8.1% and 6.5% respectively. Aguemon et al. (2018) reported protein and moisture contents of 7.8% and 6.2% respectively for the full-fat flour of the ackee seed. It is not known whether the seeds were dehulled.

3.2 Chemical Composition of Ackee Seed Protein Isolate

As showed in Table 2, the purity (protein content) of the protein isolate from ackee seeds (75-76%) was lower than the commercial soybean protein isolate (85 - 88%). The Kjeldahl and Biuret methods gave similar results with respect to purity of the protein isolates. The moisture content of the ackee seed protein (9.13%) was higher than that of the soybean protein (6.06%), but in both cases were well within the maximum of 15.5% for dried flour products (Joint FAO/WHO Codex Alimentarius Commission, 1985). Ackee seed protein isolate had significantly higher ($p < 0.05$) residual starch (6.62%) compared with soybean (0.64%). Starch is the major component (54 - 57%) of defatted ackee seed flour, and thus likely to be present in the protein isolate as a major impurity. Higher quantities ($p < 0.05$) of fat and ash were present in the soybean protein isolate (0.604% and 3.40% respectively) compared with the ackee seed protein isolate (0.289% and 2.39% respectively). The HGA equivalent of the ackee seed protein isolate averaged 106.4 ± 6.5 ppm. This is below the limit of 150 ppm set by the Bureau of Standards Jamaica (BSJ) for ackee products (Gordon et al., 2015).

Table 2: Chemical Composition of Ackee Seed Protein Isolate and Commercial Soybean Protein Isolate

Component (g/100)	Protein Isolate [*1, *2]	
	Ackee	Soybean
Protein (Purity) – Kjeldahl	74.95 ± 2.33 [a]	85.69 ± 0.38 [b]
Protein (Purity) – Biuret	75.71 ± 1.36 [a]	87.72 ± 0.56 [b]
Moisture	9.13 ± 0.14 [b]	6.06 ± 0.34 [a]
Starch	6.62 ± 0.20 [b]	0.64 ± 0.024 [a]
Crude Fat (Pet. ether extract)	0.289 ± 0.009 [a]	0.604 ± 0.053 [b]
Ash	2.39 ± 0.05 [a]	3.40 ± 0.08 [b]

[*1]: N = 3
[*2]: Values sharing at least one letter in a row are not significantly different (95% CI)

No information regarding the chemical composition of ackee seed protein isolate have been previously reported. Rapeseed, peanut and beach pea protein isolates have been reported to have purities of 70.8%, 77.8-85.7% and 85-86%, respectively (Yoshie-Stark et al., 2008; Yu et al., 2007; Chevan et al., 2001). The purity of the protein isolates from ackee seed and soybean protein isolates were within these range reported.

3.3 Amino Acid Composition

The commercial soybean protein had greater quantities of amino acids (83.71%) compared with the ackee seed protein (69.80%). Ackee seed protein had significantly higher quantities of glycine (5.97 g/100 g) and cysteine (2.68 g/100 g), and similar quantities of arginine, threonine, tyrosine, methionine and lysine compared with soybean (see Table 3). Glutamic acid/glutamine was present in largest quantities accounting for 23% and 21% of the total amino acids in ackee seed and soybean respectively. The relatively high lysine content of ackee seed protein suggests it could be used to fortify cereal products.

Although no published information is available regarding the amino acid composition of ackee seed protein isolate, the profile presented here is mostly similar to those reported for ackee seed full-fat flours. Glutamic acid was present in highest amounts (< 10%), with moderate amounts (3–6%) of the essential amino acids leucine, isoleucine, lysine, phenylalanine and

Table 3: Amino Acid Composition of Ackee Seed Protein Isolate, Defatted Flours and Commercial Soybean Protein Isolate

Component (g/100)	Protein Isolate [*1, *2]	
	Ackee	Soybean
Aspartic Acid (Asp)[*3]	5.31 ± 0.16 [a]	9.30 ± 0.39 [b]
Glutamic Acid (Glu)[*4]	16.05 ± 0.34 [a]	17.86 ± 0.81 [b]
Serine (Ser)	3.04 ± 0.07 [a]	4.58 ± 0.19 [b]
Histidine (His)	1.58 ± 0.02 [a]	2.63 ± 0.12 [b]
Glycine (Gly)	5.97 ± 0.22 [b]	3.48 ± 0.16 [a]
Arginine (Arg)	6.41 ± 0.13 [a]	6.54 ± 0.30 [a]
Threonine (Thr)	3.03 ± 0.05 [a]	3.26 ± 0.15 [a]
Alanine (Ala) + Proline (Pro)	4.45 ± 0.08 [a]	8.01 ± 0.38 [b]
Tyrosine (Tyr)	2.88 ± 0.09 [a]	3.50 ± 0.47 [a]
Valine (Val)	2.91 ± 0.03 [a]	3.70 ± 0.27 [b]
Methionine (Met)	1.37 ± 0.04 [a]	1.31 ± 0.08 [a]
Cysteine (Cys)	2.68 ± 0.09 [b]	1.38 ± 0.13 [a]
Leucine (Leu)	2.33 ± 0.02 [a]	3.77 ± 0.32 [b]
Isoleucine (Ile)	4.41 ± 0.09 [a]	6.40 ± 0.44 [b]
Phenylalanine (Phe)	2.51 ± 0.03 [a]	4.42 ± 0.54 [b]
Lysine (Lys)	4.86 ± 0.16 [a]	5.41 ± 0.33 [a]
TOTAL	**69.80 ± 1.25 [a]**	**83.71 ± 2.45 [b]**

[*1]: N = 3
[*2]: Values sharing at least one letter in a row are not significantly different (95% CI)
[*3]: Includes asparagine (Asn)
[*4]: Includes glutamine (Glu)

valine (Abiodun et al., 2015; Adeyeye, 2011; Djenontin et al., 2009). Higher quantities (2.7%) of the essential amino acid cysteine are being reported in this study.

3.4 Water and Oil Absorption Capacities

Soybean protein isolate had significantly higher water absorption capacity (6.92 ± 0.11 g H_2O/g isolate) when compared with ackee seed protein isolate (1.88 ± 0.13 g H_2O/g isolate). This suggests that soybean protein has more surface polar amino acids to bind more water molecules (Zayas, 1997). An alternative explanation is the much lower solubility of the soybean protein isolate compared with ackee seed protein (Section 3.4); insoluble proteins have been reported to have higher water binding capacities than more soluble proteins (Culbertson, 2005). Zayas (1997) reported water absorption capacity of soybean protein isolates ranging from 6.0 to 11.3 ml H_2O/g protein.

Water absorption capacity of ackee seed protein isolate has not been previously reported, however, Akintayo et al. (2002) reported values of 1.30 g H_2O/g flour for the full-fat flour. This is lower than that reported in this study for the ackee protein isolate. Khalid et al. (2003) stated that water absorption of commercial protein isolates typically ranged from 1.90 - 2.90 g H_2O/g isolate. Peanut concentrates, and cowpea concentrates have been reported to have water absorption of 1.2-2.7 g/g and 0.85-1.73 g/g, respectively (Mune et al., 2014). Ghribi et al. (2016) reported values for chickpea concentrates ranging from 2.1 - 2.7 g/g. These values are similar to that reported in this study for ackee seed protein isolate.

Oil absorption capacity of ackee seed protein (1.22 ± 0.06 g oil/g isolate) was significantly higher than that of soybean (0.86 ± 0.06 g oil/g isolate). This implies that the ackee seed proteins have more exposed hydrophobic amino acids, and/or the physical structural matrices of the ackee proteins allow for greater oil entrapment compared with soybean (Zayas, 1997). No prior data exist regarding oil absorption of ackee seed protein isolate. Akintayo et al. (2002) reported values for the full-fat flour of 1.25 g oil/g flour, which is similar to values reported for the protein isolate in this study. Reported oil absorption of soybean protein isolates ranged from 1.2 to 1.9 ml oil/g protein (Zayas, 1997; Mune et al, 2014). Oil absorption capacities of 1.88 and 1.55 ml/g have been reported for quinoa and wheat respectively (Elsohaimy et al., 2015).

3.5 Solubility of Ackee Seed Protein Isolate in Britton-Robinson Buffer

The solubility profile of ackee seed protein isolate in the buffer at 0.1 M ionic strength follows the classical pattern displayed by most native proteins (see Figure 2). Solubility was highest at acidic and alkaline pH values but fell to a minimum at pH 4.5, which is within the isoelectric pH region. The solubility of ackee proteins was greater than 70% even within the isoelectric pH region. Proteins that remain soluble at their isoelectric point contain large numbers of surface polar amino acid residues when compared with non-polar amino acid residues (Damodaran, 1997). Greater surface hydrophilicity means that there are interactions between protein molecules and solvent outweigh protein-protein hydrophobic interactions, hence the proteins remain in solution (Damodaran, 1997).

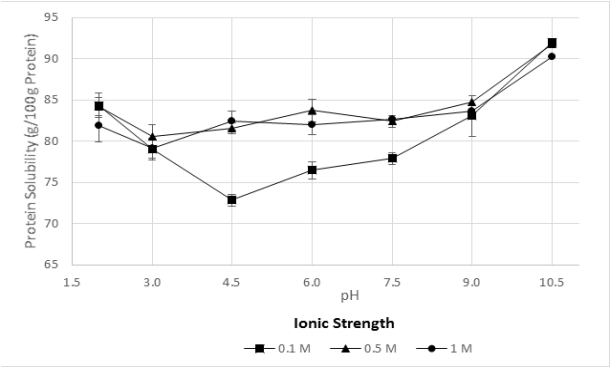


Figure 2: Effect of pH and Ionic Strength on the Solubility of Ackee Seed Protein Isolate

Protein solubility increased further around the isoelectric pH region at higher ionic strengths (0.5 M and 1.0 M). The salt ions may have altered the surface charge on the protein molecules causing them to no longer exhibit net neutrality at their isoelectric point, thus increasing solubility (Sathe, 2012). Chavan et al. (2001) also reported that the addition of NaCl increases the solubility of beach pea protein around its isoelectric point. At pH 4.5, 6.0 and 7.5, it was observed that the solubility of ackee seed protein increased as ionic strength was increased from 0.1 M to 0.5 M; further increase in the ionic strength to 1.0 M did not increase the solubility further.

Ackee seed proteins were more soluble in the Britton-Robinson Buffer when compared with the commercial soybean protein under all pH/ionic strength conditions tested. Solubility of the soybean protein

isolate ranged from 1.96% (pH 4.5, 0.1 M) to 20.17% (pH 10.5, 0.1 M). When ionic strength was increased, the solubility decreased further. The commercial soybean protein may have been denatured during the isolation process. Reduced solubility is a direct consequence of protein denaturation (Pelegrine and Gasparetto, 2005). Lee et al. (2003) reported soybean protein isolates having high solubility from pH 2.0 to 12.0, those exhibiting low solubility at pH 4.3 but became more soluble as conditions became more alkaline, and the final group that shows low solubility at all pH levels from 2.0 to 12.0. The soybean isolate used in this study appears to fall in the latter category.

The high solubility of ackee seed protein isolate suggests that the proteins were still in their native state, and are suited for fortification of acidic fruit juices and dairy products such as yoghurts, especially since solubility remain high within the 3.0-5.0 pH range. High protein solubility (> 70%) was also reported for pea isolate (Adebiyi and Aluko, 2011) and ultra-filtered rapeseed protein isolate (Yoshie-Stark et al., 2008). The solubility of ackee seed protein isolate has never been previously reported, but Akintayo et al. (2002) reported solubility values ranging from 40 – 60% (pH 2 – 10) for the full-fat flour. The ackee protein isolate was therefore more soluble than the flour.

3.6 Emulsion Capacity

As showed in Figure 3, the emulsion capacity (%) of ackee seed protein isolate was significantly affected by pH x ionic strength interaction ($p < 0.05$). Emulsion capacity was relatively low at pH 4.5 (0.1 M ionic strength), pH 9.0 (1.0 M) and pH 10.5 (0.5 M and 1.0 M). The low emulsion capacity at pH 4.5 (25.79 ± 2.72%) can be attributed to the lower protein solubility at this pH when the ionic strength was 0.1 M. Emulsion capacity of the ackee seed proteins was significantly higher at pH 4.5 ($p < 0.05$), when the ionic strength was increased to 0.5 M and 1.0 M. This was as a result of increased solubility at the isoelectric pH range when the ionic strength was increased. Proteins must first be solubilised in order to migrate from the bulk aqueous phase to the oil-water interface where the free energy is lower (Damodaran, 1997; Adebayo et al., 2013).

Additionally, an increase in ionic strength produces charged layers around fat droplets and lowers interfacial energy, resulting in increased repulsion among droplets and delayed coalescence (Adebayo et al., 2013; Deng et al., 2011). The emulsion capacity of ackee seed protein decreased when the pH was 10.5, particularly at higher ionic strengths, despite being more soluble at alkaline pH levels. Extremes of pH tend to reduce the emulsifying capabilities of proteins since they become highly charged and do not interact with the dispersed phase to form flexible cohesive films (Culbertson, 2005).

Regardless of ionic strength, ackee seed protein isolate appears to be a good emulsifying agent at pH 2.0 (47-57%), pH 3.0 (47-51%), pH 6.0 (43-51%) and pH 7.5 (53-58%). The commercial soybean protein isolate used in this study did not produce any emulsion under similar conditions. Emulsions were formed only at pH 9.0 (0.1 M) and pH 10.5 (0.1 M and 0.5 M). Under those conditions, the emulsion capacity was 49.97 ± 1.96%, 60.80 ± 3.76% and 33.78 ± 1.99%, respectively. Alkaline conditions appear to enhance the ability of soybean protein to form emulsions.

The emulsion properties of ackee seed protein isolate have not been previously reported. Akintayo et al. (2002) reported a value of 25.6% for the full-fat flour. This value is lower than that of the ackee protein isolate reported in this study. Chel-Guerrero et al. (2002) reported that the legumes *Phaseolus lunatus* and *Canavalia ensiformis* had emulsion capacity ranging from 41-56% and 48-52%, respectively. Pedroche et al. (2004) reported that *Brassica carinata* protein isolates extracted at pH 10.0, 11.0 and 12.0, had emulsion capacity values of 70%, 54% and 15%, respectively.

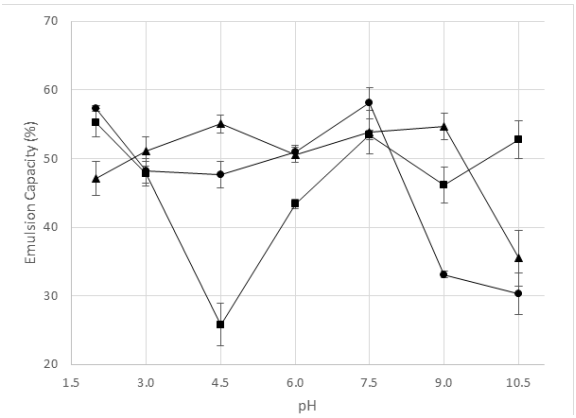


Figure 3: Effect of pH and Ionic Strength on Emulsion Capacity of Ackee Seed Protein Isolate

3.7 Emulsion Stability

Emulsions are thermodynamically unstable, and the phases will spontaneously separate resulting in creaming, flocculation, coalescence or phase inversion (Hill, 1996). The stability (%) of the emulsions formed by ackee seed protein was significantly affected by pH and ionic strength. At the lowest ionic strength (0.1 M), emulsion stability was relatively high (approximately 90%) within the pH range of 2.0 to 10.5, except pH 6.0 (see Figure 4), where there was a significant reduction in the emulsion stability.

At higher ionic strengths (0.5 M and 1.0 M), emulsion stability decreased at pH 4.5 which is within the isoelectric region. Emulsion stability also decreased at pH 9.0. As conditions became more alkaline at pH 10.5, the emulsions completely broke down when heated. This was indicative of further instability. The alkaline pH, along with high salt concentration, caused the protein molecules to become highly charged so they do not form strong stabilising forces with the dispersed phase (Culbertson, 2005). This increases the likelihood of phase separation and reduced emulsion stability (Culbertson, 2005). For the commercial soybean protein, stable emulsions only formed at pH 9.0 and

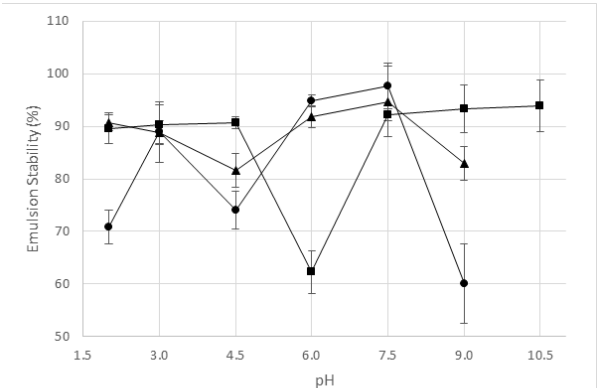


Figure 4: Effect of pH and Ionic Strength on Emulsion Stability of Ackee Seed Protein Isolate

10.5 with emulsion stability values ranging from 79-99%.

In this study, highest emulsion stability (> 90%) was recorded at pH 2.0 (0.1M and 0.5M), pH 3.0, pH 4.5 (0.1M), pH 6.0 (0.5M and 1.0M), pH 7.5 and pH 9.0 (0.1M) for ackee seed protein. The emulsion stability of ackee seed proteins, whether in the form of isolate or flour, has not been previously reported. Deng et al. (2011) reported that *Ginkgo biloba* seed protein isolate had emulsion stability ranging from 78-90%. Emulsion stabilities of 90 - 100% have been reported for *Phaseolus lunatus* and *Canavalia ensiformis* at acidic pH (2.0 - 4.0) and alkaline pH (8.0 - 10.0). Relatively low emulsion stabilities have been reported for protein isolates of mung bean (15%) (El-Adway, 2000) and *Brassica carinata* (33%) (Pedroche et al., 2004). The emulsion capacity and stability of ackee seed protein were relatively high when compared with values reported for other proteins. Ackee seed protein may thus be suitable for use as an emulsifying agent in foods such as margarine and butter, mayonnaise, and ice cream, and particularly suited for use in high-acid foods.

3.8 Foaming Capacity

The foaming capacity (% overrun) of ackee seed protein isolate increased significantly ($p < 0.05$) as ionic strength increased. This pattern was observed at all pH levels tested. The interaction between pH and ionic strength was significant primarily because the difference in percentage overrun among the levels of ionic strength tested was unequal at different pH levels (see Figure 5). Foaming capacity of ackee seed protein was highest at acidic pH levels (2.0-4.5), however, at pH 6.0, foaming capacity was significantly lower ($p < 0.05$). Foaming capacity did not significantly improve as pH conditions became more alkaline, with the exception of pH 7.5 (1.0 M). Ackee seed protein therefore has a better foaming capacity under acidic conditions.

The highest foaming capacity of ackee seed protein was at pH 2.0-4.5 and ranged from 106-119% (0.1 M ionic strength), 142-151% (0.5 M) and 162-201% (1.0 M). The foaming capacity of ackee seed protein isolate has not been previously reported but Akintayo et al. (2002) reported values of 27.1% for the full-fat ackee seed flour in distilled water. Comparable foaming capacity has been reported for proteins from egg albumin (156-200%), pigeon pea (110-130%), beach pea (128-143%) and lupin peas (104-106%) (El-Adawy, 2001; Chavan et al., 2001; Lomakina and Mikova, 2006; Eltayeb et al., 2010).

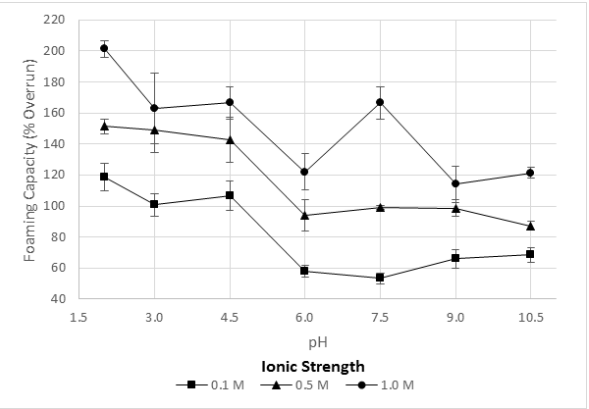


Figure 5: Effect of pH and Ionic Strength on Foaming Capacity of Ackee Seed Protein Isolate

Many studies report an increase in foaming capacity and stability at extreme acidic pH levels (pH 1.0-2.0) and more so at alkaline pH levels (pH 8.0-12.0). The pH range 4.0-6.0 lies within the isoelectric pH range of most proteins and values for foaming capacity and stability are usually lower. This phenomenon has been reported for proteins from sesame seed, cowpea and pumpkin seed (Ragab et al., 2004; Lazos, 1992; Khalid et al., 2003). For ackee seed protein, the relationship between pH and foaming capacity/stability was somewhat different. Highest values were recorded at acidic pH levels (2.0, 3.0 and 4.5). Both foaming capacity and stability were lower at alkaline pH values despite increased protein solubility. Reductions of foaming capacity at alkaline pH have also been reported for protein isolates of pigeon pea and cashew nut (Ogunwolu et al., 2009; Eltayeb et al., 2010). This has been attributed to the repulsion of peptides via ionic repulsions (Klompong et al., 2007; Ogunwolu et al., 2009). Unlike many food proteins, ackee seed protein isolate showed high foaming capacity around its isoelectric pH region (4.0-4.5). A likely reason is that the solubility of the protein remains high even within the isoelectric pH range. Soluble proteins are said to have maximum foaming capacity at pH close to their isoelectric point (Zayas, 1997).

The foaming capacity of ackee seed protein was significantly higher ($p < 0.05$) than soybean protein. This was evident at all pH and ionic strengths tested, particularly at acidic pH levels (see Figure 6). When the foaming experiment was carried out using distilled water, the foaming capacity of ackee seed protein ($40.67 \pm 1.15\%$) was significantly higher than soybean ($20.67 \pm 1.15\%$). A likely factor for the lower foaming capacity of the soybean isolate is its poor solubility.

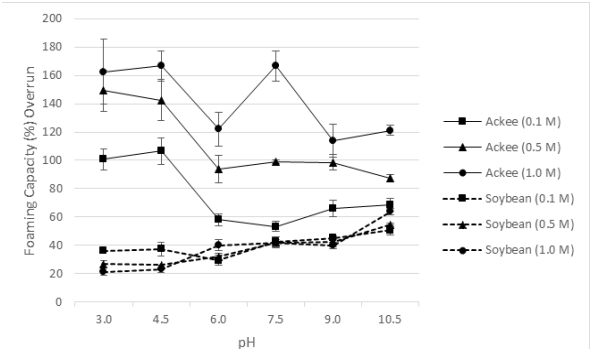


Figure 6. Foaming Capacity of Protein Isolates from Ackee Seed and Soybean

3.9 Foam Stability

Foam stability (%) was lowest at the lowest ionic strength tested (0.1 M) at all pH levels, this effect was more pronounced within the pH range 6.0 - 10.5 (Figure 7). Foam stability improved when ionic strength was increased from 0.5 M to 1.0 M, but only within the pH range 6.0 - 10.5. Interactions between ionic strength and pH were therefore significant ($p < 0.05$). Deng et al. (2011) reported increased foam stability of *Ginkgo biloba* proteins when NaCl concentration was increased within the range 0 - 0.75 M. Damodaran (1997) stated that the foam stability of most globular proteins such as egg albumins and glutens improved by increasing NaCl concentration due to neutralisation of charges on protein molecules. This minimises repulsions and favours the formation of strong, viscous films at the air-water interface, which is necessary for stable foams (Damodaran, 1997).

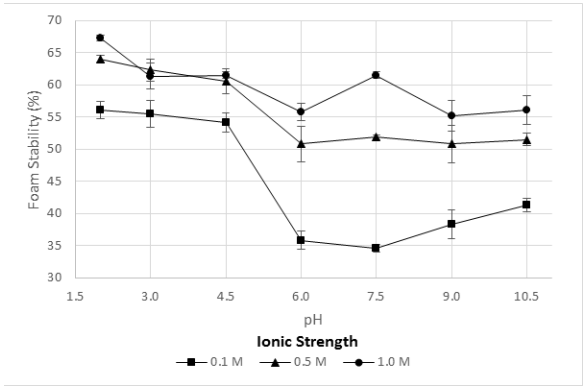
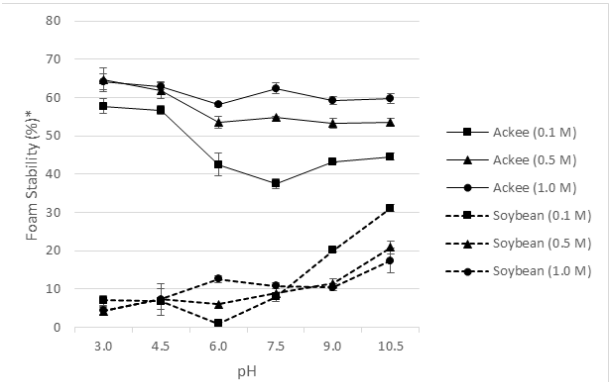


Figure 7: Effect of pH x Ionic Strength on Foam Stability of Ackee Seed Protein Isolate

Ackee seed protein foams were more stable at acidic pH (2.0-4.5) when compared with higher pH values. Foams produced at pH 4.5 were found to be most stable: 45.88% of initial foam volume (IFV) remained after standing for 24 h. This was significantly higher when compared with pH 3.0 (37.35% IFV) and pH 2.0 (22.36% IFV) after 24 h. Protein foams are most stable within their isoelectric pH range due to increased intermolecular interactions among protein molecules, which improve film thickness and strength (Zayas, 1997; Damodaran, 1997).

Ackee seed protein isolate demonstrated higher foaming stability (37 - 64%, $p < 0.05$) compared with the commercial soybean protein isolate (1 - 31%) (see Figure 8). Whereas the foam stability of ackee seed protein decreased at alkaline pH levels, foam stability of the soybean protein increased. A likely reason for this is the increased solubility of soybean proteins at alkaline pH values (see Section 3.5). Foam stability for ackee seed protein (27.48 ± 2.81%) was also higher ($p<0.05$) than soybean (15.74 ± 0.42%) when the experiment was done using distilled water. The foam produced by ackee seed protein was denser and more viscous than soybean foam (see Figure 9).



*: % of initial foam volume remaining after 1 h

Figure 8: Foam Stability of Protein Isolates from Ackee Seed and Soybean

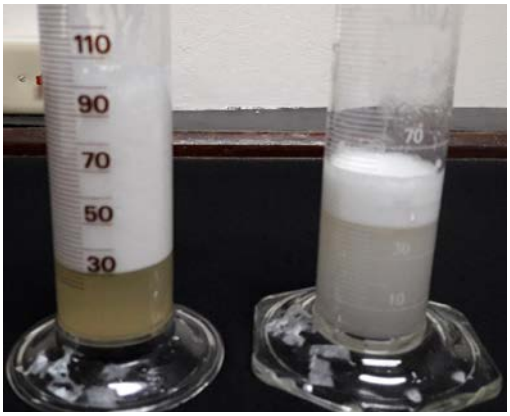


Figure 9: Protein Foams – Ackee Seed (Left); Soybean (Right)

Ackee protein foams therefore have greater film thickness and hence greater mechanical strength and viscoelastic properties compared with soybean foams (Zayas, 1997). Ackee seed protein films would be thus more impermeable to trapped air, drain less liquid and shows greater resistance to mechanical stresses such as expansion and compression compared with soybean films (Zayas, 1997; Chavan et al., 2001). The foam stability of ackee seed protein is comparable to those reported for egg albumin (33 - 54%, 1h) (Lomakina and Mikova, 2006), quinoa (54.54%, 1h) (Elsohaimy et al., 2015) and cashew nut (55%, 1h) (Ogunwolu et al.,

2009). Akintayo et al. (2002) reported that the ackee seed full-fat flour had a foam stability of 11.4% after standing for 2 hours. This value is much lower than those reported in this study for the protein isolates.

Ackee seed protein isolate has the potential for use as a foaming agent in foods such as juices, milkshakes, marshmallows and beer, and is particularly suited for use as a foaming agent in acidic fruit juices and milk products.

3.10 Protein Molecular Weights

Ackee seed protein isolate was found to have lower molecular weight (kDa) proteins compared with soybean (Figure 10). For the ackee protein isolate, six bands were observed having molecular weights (kDa) of 29.80 ± 1.86, 23.77 ± 0.75, 14.51 ± 0.34, 11.98 ± 0.17, 10.44 ± 0.06, and 8.73 ± 0.48. The four latter bands, corresponding to molecular weights less than 12 kDa, were more intense. This suggests that these proteins were present in greater proportion compared with the two faint bands corresponding to the higher molecular weights. For the soybean isolate, eight bands were observed with molecular weights (kDa) of 101.31 ± 3.41, 85.13 ± 2.50, 77.46 ± 0.15, 54.28 ± 0.49, 44.18 ± 0.52, 37.40 ± 0.79, 21.04 ± 0.53, and 12.67 ± 0.37. The most intense bands corresponded to molecular weights (kDa) of 77.46, 37.40 and 21.04. Nwozo et al. (2014) reported that a trysin inhibitor protein fraction of ackee seed had molecular weights between 20 – 30 kDa, this corresponds to the two heavier bands reported in this study.

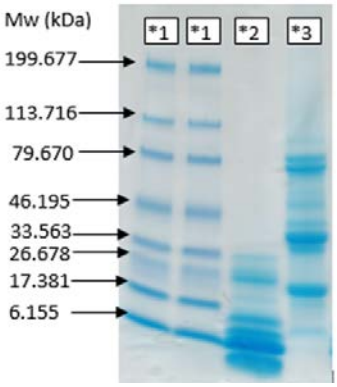


Figure 10. Electropherogram of Ackee Seed and Soybean Proteins

The lower molecular weights of ackee seed proteins compared with soybean proteins may also account for the superior functional properties of the ackee seed proteins. For instance, the high foamability of ackee seed proteins can be related to molecular weight, since low molecular weight proteins and peptides can diffuse faster to the air-water interface and produce more foams (Wilde and Clark, 1996). Small molecular weight proteins are more water-soluble because they have reduced hydrophobicity and more exposed polar residues which results in more hydrogen bonds being formed with the aqueous solvent molecules (Chabanon et al., 2007; Dong et al., 2008). Many studies have focused on improving the functional properties of protein isolates and flours by hydrolysing the proteins into smaller molecules and peptides, using enzymes such as pepsin, trypsin and chymotrypsin (Wilde and Clark, 1996; Damodaran, 2005). Ackee seed proteins have low molecular weights and high functionalities therefore hydrolysis might not be necessary.

4. Conclusion

The ackee seed protein isolate was highly soluble even within its isoelectric pH range. Generally, solubility increased as ionic strength increased. The low molecular weights of the ackee seed protein accounted for its high solubility, which in turn accounted for its superior emulsification and foaming properties compared with other plant proteins previously reported.

Ackee seed protein showed good emulsification and foaming properties at acidic pH range (3.0-5.0). Literature data for other plant proteins revealed poor functionalities at acidic conditions. Ackee seed protein, therefore, can be utilised in acidic foods such as fruit juices, yoghurt, sour milk and other high-acid foods. The water and oil absorption capacities of ackee seed protein were moderate when compared with other proteins. Ackee seed protein showed superior functionalities compared with the commercial soybean protein isolate, however, water absorption and purity values were higher for the soybean protein. The principal amino acids of ackee and soybean proteins were glutamic acid, aspartic acid and arginine.

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