STUDIES ON THE PROXIMATE COMPOSITION, FUNCTIONAL PROPERTIES AND EFFECT OF pH AND SALT CONCENTRATIONS ON SOME FUNCTIONAL PROPERTIES OF *ACKEE* APPLE ARIL FLOURS

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ABSTRACT

The present study produced full fat and defatted flours from matured ackee apple arils and evaluated the proximate composition and the effect of pH and salt concentrations on some of the functional properties of the full fat flour and the defatted flour. Matured ackee apple arils were oven-dried at 60 ^{0}C and milled to obtain dried arils whole flours. The flour was divided into two portions: The first portion was subjected to cold acetone extraction at room temperature (28 ± 2^{0}C). The residual solvent in the sample was evaporated at room temperature to obtain the dried defatted flour (ADF). The second portion was taken as full fat flour (AWF). Proximate composition, functional properties and effect of pH and salt concentration on some functional properties were evaluated. The results of the proximate composition showed that the protein content of ADF was 36.43% while that of AWF was 20.13%. The bulk density, water absorption and oil absorption capacities, foaming properties and emulsifying properties of ADF were also observed to be higher than that of AWF. The solubility profile of the flours showed that ADF was more soluble than AWF and both samples were least soluble at pH 4 and the highest solubility was observed at pH 10. Increase in salt concentrations of the sample solutions was observed to increase some of the functional properties of the sample at each of the pH values considered. The study concluded that ackee apple flours could find applications as functional ingredient in food.

Keywords: Ackee apple, Proximate composition, Functional properties, Protein solubility

INTRODUCTION

Until now, the bulk of the protein for human food in many African countries are sourced from oil seed such as soybeans. Meanwhile, a wide range of tree plants containing fruits that are rich in proteins exist in the forest of many African countries which are underutilized (Ezeagwu *et al.,* 2004). The under-utilization of some of these protein tree bearing fruits is due to limited information on their health and nutritional benefits. One of such fruits is ackee apple.

Ackee apple, scientifically called *Blighia sapida* and locally called *isin* is of the family *Sapindaceae*. The ackee apple tree is an evergreen tree more widely known for the edible part of its fruit called arils. The origin of the fruit has been traced to Jamaica where the edible part (arils) form part of their national dish (Oyeleke *et al*., 2014). The ripe arils of the ackee fruit, are yellow to cream-coloured and are nutty in flavour. In some West African countries, ripe fruit arils are eaten fresh, dried, fried, roasted or made into sauce or soup.

Salt concentrations and the level of acidity or alkalinity of solutions are important factors influencing some functional properties such as foaming, emulsifying properties and solubility of any protein-containing materials. Substantial scientific evidence exists on the beneficial effect of ackee apple aril flours. The aril of the fruit has been reported to be rich in protein, vitamin C and phytochemical. A search through the literature revealed a dearth of information on the functional behaviour of the ackee apple aril flour with respect to pH and salt concentrations.

This study, therefore, aims to investigate the proximate compositions and how salt concentrations and pH affect some of the functional properties of the whole and defatted ackee apple arils. This will further enhance the utilization of the fruit as a functional food ingredient.

MATERIALS AND METHOD

Collection of Materials

Matured ackee apple fruits were gathered from ackee apple trees at the botanical garden of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Preparation of whole flour and defatted flours of ackee apple arils

The arils were separated from the ackee pods and seeds manually. The arils were washed to remove unwanted materials and dried using hot air oven (Uniscope SM9053 Laboratory Oven, Surgifriend Medicals, England) maintained at a temperature of 55 ± 2 °C for 8 hr. The dried arils were milled using warring blender (BLG-450, Binatone, Shenzhen, China) set at the highest speed to obtain ackee apple aril full fat flour (AWF). Defatted flour was obtained from the full fat flour using the acetone extraction method at room temperature. About 200 g of the whole flour was suspended into two litres of acetone to give a ratio of 1:10 (w/v). The suspension was stirred on a magnetic stirrer (Breda, C/S 50, Netherlands) for 4 hr. The suspension was then passed through muslin cloth to separate the residue from the supernatant. The residue was thinly spread at room temperature to evaporate the remaining solvent from the flour. The defatted flour was then finely ground using warring blender (BLG-450, Binatone, Shenzhen, China) to obtain homogenous defatted flour (ADF).

CHEMICAL ANALYSIS

Proximate Composition

Moisture content, Ash content, Crude Fibre, Crude Protein and Carbohydrate contents were determined according to official method of Association of Analytical Chemist (AOAC, 2000)

Determination of the Physicochemical and Functional Properties

Bulk Density

Bulk density was determined by the method of Okezie and Bello (1988).

pH

The pH of the samples was measured by making a 10% w/v suspension of the sample in distilled water. The suspension was mixed thoroughly and the pH value was measured with a pH meter (Model H198107, Hanna instrument, 35010 Padovana, Italy).

Water Absorption Capacity (WAC)

The WAC was determined according to the method of AACC (1995).

Oil Absorption Capacity

Oil absorption capacity of the flour samples was determined by the centrifugal method described by Beuchat (1977).

Effect of pH and NaCl Concentration on Emulsifying Activity Index and Emulsion Stability

The effect of pH and salt concentration on emulsifying activity index (EAI) was determined by the method described by Gbadamosi *et al.* (2012). The emulsion stability was determined by allowing the emulsions to stand for 10 min at room temperature and the ESI was determined as described above, and expressed as a percentage of the initial EAI (Aluko and Yada, 1993).

Effect of pH and NaCl Concentration on Foaming Capacity and Stability

Foam capacity and foam stability as influenced by pH and salt concentration were determined by a modification of the method described by Chavan *et al.* (2001).

Protein Solubility

The effect of pH on protein solubility was determined by a method described by Gbadamosi *et al.* (2011) with slight modifications. The protein content was then determined using the modified Lowry method.

Protein Determination by Lowry Method

The protein content of supernatant A and B was determined using the modified Lowry method (Markwell *et al.,* 1978).

Statistical Analysis

The data obtained in the analysis were subjected to Analysis of Variance (ANOVA) and the means were separated using Duncan multiple range test.

RESULTS

Table 1: Proximate Composition of Ackee Apple Aril Flours

	ADF	AWF
Moisture (%)	3.79±0.17[b]	8.67±0.26[a]
Ash (%)	2.44±0.05[b]	3.60±0.04[a]
Fiber (%)	1.69±0.12[b]	4.23±0.14[a]
Protein (%)	36.43±0.29[a]	20.13±0.21[b]
Crude fat (%)	2.43±1.60[b]	35.80±0.13[a]
Carbohydrate (%)	53.22±0.67[a]	27.57±0.39[b]

Values reported are means ± standard deviation of triplicate determinations. Mean values with different superscripts within the same column are significantly ($P < 0.05$) different

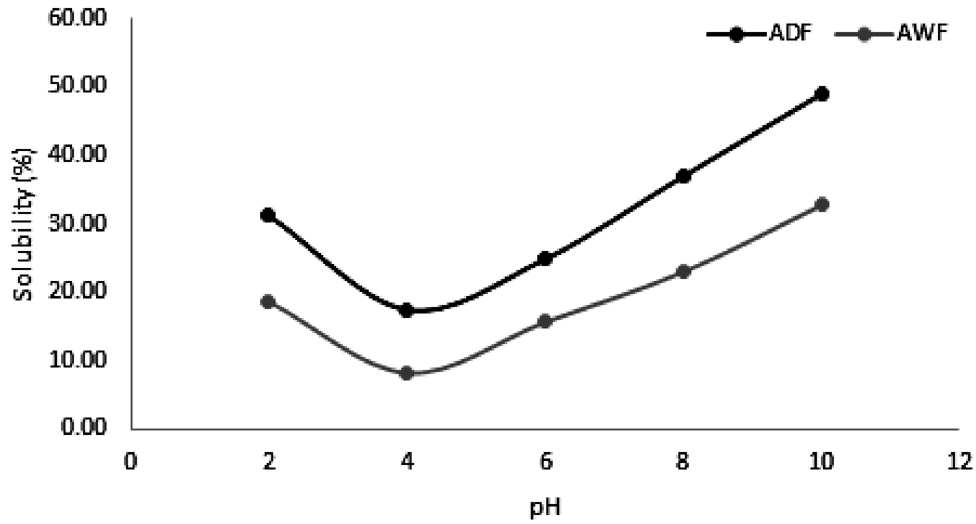
ADF: Ackee apple defatted flour, **AWF**: Ackee apple aril full fat flour

Table 2: Functional Properties of Ackee Apple Flours

	ADF	AWF
pH	5.6±0.02[b]	6.4±0.01[a]
Bulk density (mg/mL)	0.72±0.08[b]	0.89±0.03[a]
Water absorption capacity (%)	302.33±0.13[b]	232.94±1.75[a]
Oil absorption capacity (%)	114.83±1.23[a]	104.43±1.01[b]
Foaming capacity (%)	8.75±0.60[a]	5.65±0.13[b]
Foaming stability (%)	58.33±0.97[a]	27.57±0.85[b]
Emulsifying activity index (m^2/g)	287.88±2.17[a]	234±1.07[b]
Emulsifying stability index (%)	111.41±1.07[a]	97.43±1.17[b]

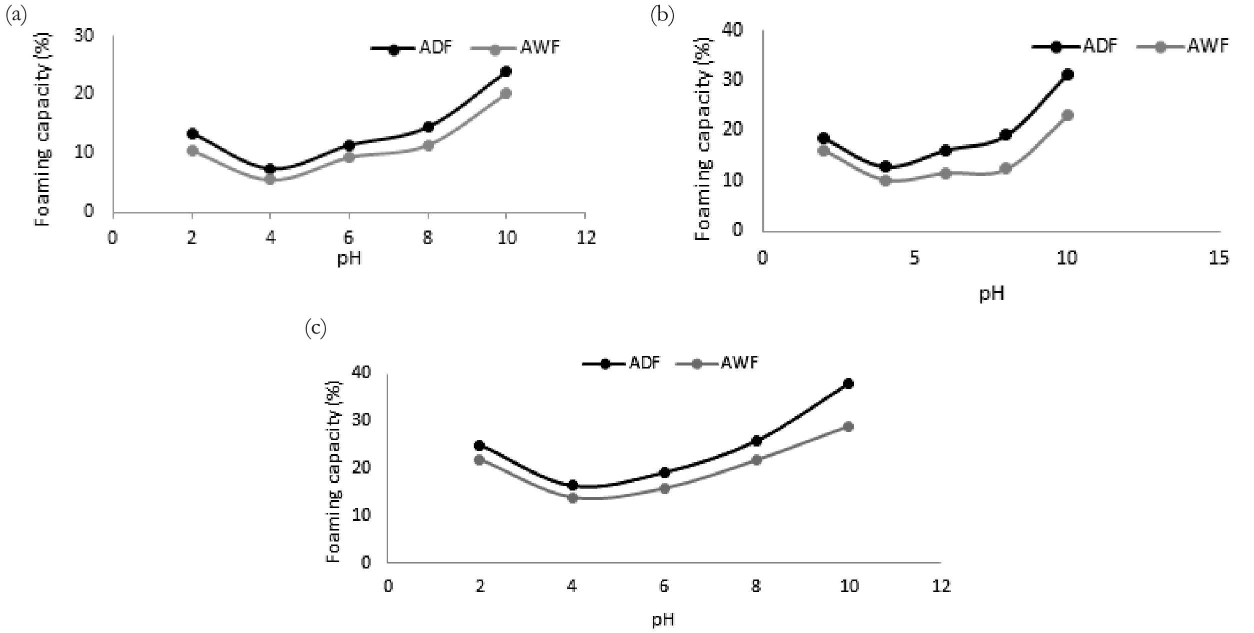
Values reported are means ± standard deviation of triplicate determinations. Mean values with different superscript within the same column are significantly ($P < 0.05$) different

ADF: Ackee apple defatted flour, **AWF**: Ackee apple full fat flour



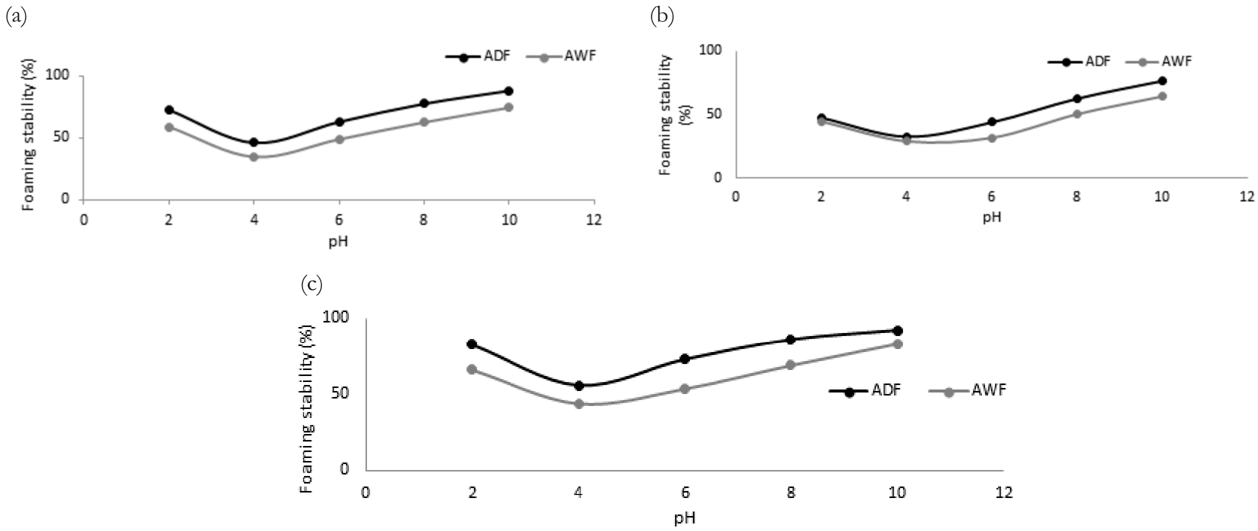
ADF: Ackee defatted flour, **AWF**: Ackee full fat flour

Figure 1: Solubility Curve at Different pH of the Flour Samples



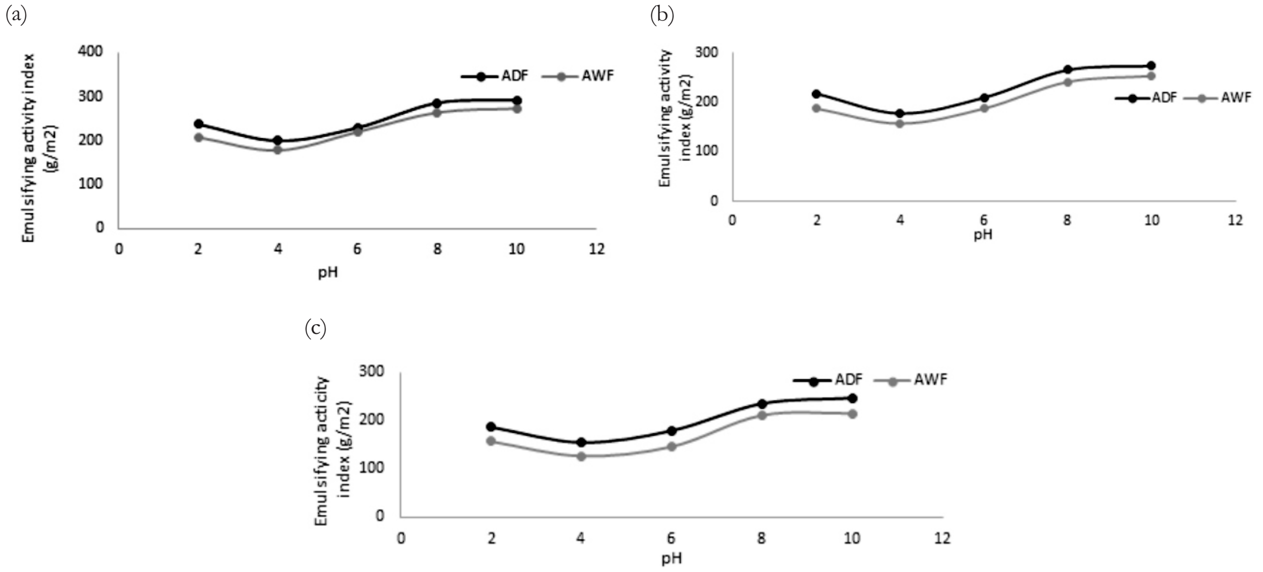
ADF: Ackee deffated flour, **AWF:** Ackee full fat flour

Figure 2: Effect of pH on foaming capacity of Ackee apple flours at (a) 0.0 M (b) 0.5 M (c) 1.0 M NaCl concentration



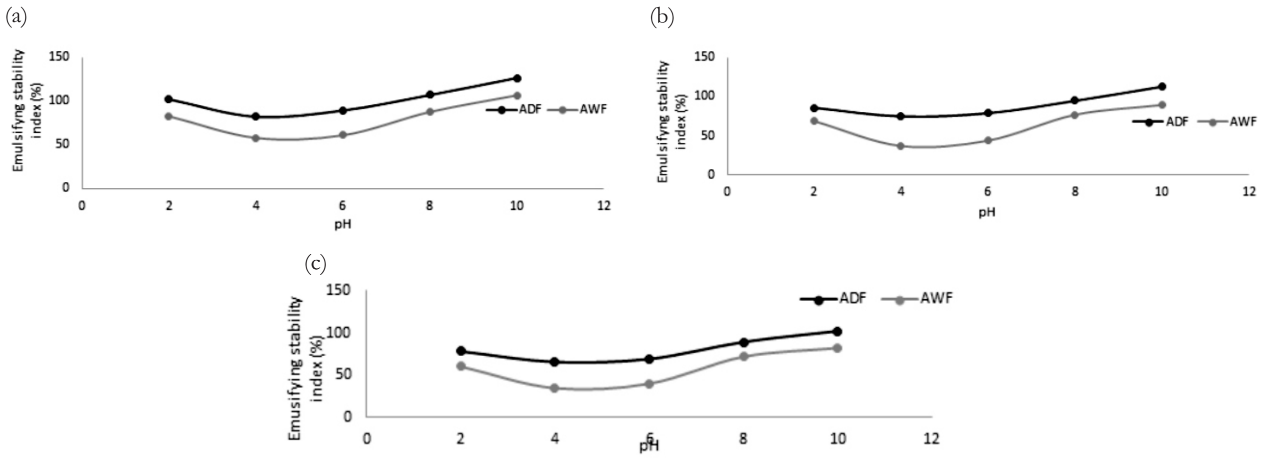
ADF: Ackee Defatted Flour, **AWF**: Ackee Full Fat Flour

Figure 3: Effect of pH on Foaming Stability of Ackee Apple Flours at (a) 0.0 M (b) 0.5 M (c) 1.0 M NaCl Concentration



ADF: Ackee Defatted Flour, **AWF**: Ackee Full Fat Flour

Figure 4: Effect of pH on Emulsifying Activity Index of Ackee Apple Flours at (a) 0.0 M (b) 0.5 M (c) 1.0 M NaCl Concentration



ADF: Ackee Defatted Flour, **AWF**: Ackee Full Fat Flour

Figure 5: Effect of pH on Emulsifying Stability Index of Ackee Apple Flours at (a) 0.0 M (b) 0.5 M (c) 1.0M NaCl Concentration

DISCUSSION

Proximate Composition of Ackee Apple Aril Flours

The proximate composition of food samples shows the level of some available basic nutrients in such food product. The ash content and protein content of food are indications of the level of mineral elements and amino acid content respectively in such food samples. These compositions were evaluated on the flours of ackee apple arils using established procedures. The moisture content of the ackee apple aril flours are shown in Table 1. Results showed that AWF had higher moisture content than the ADF. The ash content which is a measure of the level of minerals in the samples were also found to be higher in AWF than ADF. The crude fibre content of the samples was found to be significantly higher in AWF than ADF. Sample AWF was also found to contain higher fat content than ADF while the protein content of ADF was significantly higher than AWF. The values obtained for crude fat content, protein and carbohydrate obtained in this work were slightly higher than the values reported earlier in literature for oven-dried and freeze-dried flours by Dossou *et al.* (2014) but lower than those reported by Oyeleke *et al.* (2013). The differences observed in the crude protein and fat contents of the samples in this study is due to defatting process involved in the production of sample ADF. During this process, some soluble minerals are removed; the fat is removed thereby increasing the protein content of the defatted flour.

Functional Properties of Ackee Flour Arils

The hydrogen ion concentration (pH) of the ackee apple aril flours is found in Table 2. The pH value of sample ADF was 5.6 and significantly lower than the value obtained for sample AWF which was 6.4. Gbadamosi *et al.* (2008) reported similar value (6.03) for conophor defatted flour. The values obtained showed that the defatted flour was more acidic than the full fat flour. The reason might be due to the concentration of protein in the sample ADF than sample AWF. The pH of protein flour is important as it influences some functional properties of proteins such as emulsion properties and whipability.

The bulk density of sample ADF was found to be 0.72 g/mL and this value was lower than 0.89 g/mL obtained for sample AWF. The bulk density of any food material is a function of the combined effect of interrelated factors such as density of the inter-particle forces and particle sizes (Peleg and Bargley, 1993). The values obtained in this work was higher that the values for conophor defatted flour (0.41 g/mL) reported by Gbadamosi *et al.* (2008) and also higher than the value reported for soy flour (0.48 g/mL) by Okezie and Bello (1988). The higher bulk densities of the samples in this study are higher than the values reported for some oil seeds in the literature. High bulk density is desirable as it offers greater packaging advantages because greater quantities can be packed within a constant volume. The lower bulk density observed for ADF compared with AWF could be attributable to degradation of some high molecular weight compounds during the defatting process.

Water absorption capacity of sample ADF was found to be 302.33 % while 232.94 % was obtained for sample AWF. The water absorption capacity of protein sample is a function of several parameters including conformational characteristics, hydrophilic-hydrophobic amino acid balance in the protein molecule as well as lipids (Chavan *et al.*, 2001). The water absorption capacity of aril flours obtained in this work were higher than the values earlier reported by Akintayo *et al.* (2002) for ackee aril flours and also higher than the values obtained for defatted conophor flours by Gbadamosi *et al.* (2012). The high water absorption capacity of ackee apple flours confer on it the potential to be as ingredient in the fortification of bread and cake in industries.

Oil absorption capacity obtained for ADF was 114.83 % while that of AWF was 104.43 %. This trend is similar to that obtained for oil absorption capacity for the samples in this study. Kinsella (1982) established the fact that the ability of any protein sample to bind or absorb fat or oil is important as it is important for such applications as meat replacement and extenders because it enhances flavour retention mouthfeel. The value obtained for ackee apple aril flours in this study was lower than the value reported for *kariya* flours by Adebayo *et al.* (2013) but higher than the value reported for soy flours (84.20%) by Lin *et al.* (1974). The high oil absorption capacity of the ackee apple arils in this study would enhance its usefulness in meat industries particularly for sausages where protein samples are needed to bridge the fat and water in the product.

The results of the foam capacity obtained for ADF was 8.75% while that obtained for AWF was 5.65 %. The values obtained for the aril flours in this study was lower than the value obtained for soy flour (70 %) and sunflower (230%) as reported by Oshodi (1999). The values obtained in this study were comparable with the values earlier reported by Dossou *et al.* (2014).

The formation of foam is dependent on transportation, penetration and re-organizations of protein molecules at the air-water interface. For protein materials to form foams, it must be capable of migrating at the air-water interface (Haling, 1981). The same trend observed for foam capacity was also recorded for foam stability where the foam formed by sample ADF (58.33%) was stable more than the form formed by sample

AWF (27.57%). The reason for the difference in the foaming properties could be attributed to the difference in level of proteins present in both samples. This observation corroborated the submission of Damodaran (1997) that foaming properties are functions of sample protein concentration in that it increases the viscosity and enhances the formation of many layers and cohesive protein films at the interface. The low foaming properties of the samples might limit the use of the aril flours as foaming agents in food product.

At the natural hydrogen ion concentration of the ackee apple aril flours, the emulsifying activity index of sample ADF was 287.88 m^2/g. The value obtained for sample ADF was much higher than the one obtained for AWF (234.07 m^2/g). These values were much higher than the values reported for wheat flour (11.70 m^2/g) and almost three times the value reported the sunflower (95.10 m^2/g) by Lin *et al.* (1974). This same trend in emulsifying activity index was observed for emulsifying stability index where the value obtained for ADF (111.41%) was greater than sample AWF (97.43%). The results obtained in this work showed that not only did the emulsion formed by the defatted flour (ADF) higher than that full fat flour (AWF), the emulsion formed was also more stable in the defatted flour than in the full fat flour. The ability of any sample to bring together two different immiscible solutions has been attributed to the nature and concentration of protein present in such samples. This is possible because protein can emulsify and stabilize the emulsion by decreasing the surface tension of the oil droplet and providing electrostatic repulsion on the surface of the oil droplet (Regena *et al.*, 2013). Lasteri *et al.* (2011) also reported that any sample with emulsifying capacity of 70% and above has potential to be used as emulsifying agent. This shows that either the full fat flour or the defatted flour of ackee apple arils could be used as ingredients in food such as mayonnaise.

Protein Solubility

Figure 1 shows the percent protein solubility of ackee apple defatted flour (ADF) and full fat flour (AWF) at different pH levels between 2 and 10. The least solubility of ADF and AWF was found to be 17.48 % and 8.25 % at pH 4, while the maximum solubility was 49.00 % and 32.76 % at pH 10 respectively. This result is an indication that ackee apple flours are acidic in nature and that the isoelectric point of the flours occurs at pH 4.0. In this result, solubility of the samples increased rapidly below and after the isoelectric point. The result also shows that in solubilizing the protein in the ackee apple aril flours, alkaline pH is preferable to acidic region and precipitating the proteins can best be done at the acidic region. This observation agreed with the submission for African locust bean as reported by Lawal (2005). The occurrence of minimum solubility of protein near the isoelectric point could be attributed to both the net charge of peptides, which increases as pH moves away from the isoelectric point, and surface hydrophobicity that promotes the aggregation and precipitation via hydrophobic interactions (Sorgentini and Wagner, 2002). Ackee apple aril flours exhibited good solubility in both acid and alkaline pH regions, and this is an important characteristic in food fortification due to increased solubilities.

Effect of pH and Salt Concentrations on Foaming Properties

The effects of pH and salt concentration on foaming capacity (FC) of ADF and AWF are presented in Figures 2 (a-c). Foaming capacity, at all salt concentrations (0.0, 0.5 and 1.0 M) decreased from pH 2-4 and thereafter increased with increasing pH. The lowest FC at 0.0, 0.5 and 1.0 M NaCl concentrations for ADF and AWF occurred at the isoelectric pH (pH 4), which agreed with the protein solubility curve (Figure 1) in this study. At the isoelectric pH, there was an increase in foaming capacity as the salt concentration increases. This effect may be due to an increase in the net charge of the protein by the addition of NaCl which weakens hydrophobic interaction and increased protein solubility and flexibility, allowing the protein to spread to air-water interface more quickly, encapsulating air particles and thus increasing foam formation as reported by (Lawal *et al.*, 2005). The foaming stability (FS) of ackee apple aril flours measured as a function of pH and NaCl concentration is presented in Figures 3(a-c). Just like the foaming capacity, the lowest FS at pH 4 and the highest was observed at pH 10 which is the point at which the protein was most soluble. The study showed that there was an increase in FS as salt concentration and pH increased from the isoelectric pH. The enhancement of FS might be due to cross linking of protein molecules and creation of films with better viscoelastic properties (Lawal *et al*.,2005). Generally, the result obtained in this study revealed that the foaming properties of all the samples were pH-dependent and performed better at alkaline region. The foam properties of the samples were observed to increase with increase in salt concentration from 0.0 M to 1.0 M NaCl, for all the pH values. However, the foaming properties of ADF were found to be better than AWF at all the pH and salt concentrations considered. Adebowale and Lawal (2003) reported that flours are capable of producing foams because proteins in flours are surface active. The improved foaming capacity and stability of ackee apple arils in the presence of NaCl will enhance its functionality and thus its application in food such as whipped toppings, cake mixes and frosting where foaming is of paramount importance.

Effect of pH and Salt Concentration on Emulsifying Properties

Figures 4 (a-c) shows the emulsifying activity index (EAI) ADF and AWF measured as a function of pH and NaCl concentrations (0.0, 0.5 and 1.0 M). At all salt concentrations (0.0 – 1.0 M), a decrease in EAI of all the samples were observed at pH 2-4, with the lowest EAI obtained around the isoelectric regions (pH 4) and these values increased with increasing pH 6-10. At 0.0, 0.5 and 1.0 M NaCl concentration the lowest EAI obtained at the isoelectric pH values for ADF were 199 m^2/g, 177 m^2/g, 154.98 m^2/g and values for AWF were 177.69 m^2/g, 154.54 m^2/g and 126.83 m^2/g respectively. The maximum EAI of ADF and AWF at 0.0, 0.5 and 1.0 M NaCl concentrations were obtained at pH 10. According to El Nasri and El Tinay (2007), good emulsifying activity of a protein is related to its high solubility and others have shown that the pH-emulsifying properties profile of various proteins resembles the pH-solubility profile (Gbadamosi *et al.,* 2012). The low EAI values observed at pH 4 agrees with the submission of Damodaran (1997) that most food proteins are sparingly soluble at their isoelectric pH, poorly hydrated, and lack electrostatic repulsive forces, they are generally poor emulsifiers at this pH. At the isoelectric region (pH 4) however, the EAI of ADF and AWF increased with increase in salt concentration from 0.0 M to 1.0 M NaCl concentrations. At pH below and above the isoelectric point, the emulsifying activity index of ADF and AWF decreased with increase in salt concentrations from 0.0 to 1.0 M. The trends were also similar to the observations of Ogungbenle *et al.* (2009) and this was attributed to the hydrodynamics of protein molecules in food system which was greatly influenced by prevalent ionic strength. Generally, defatted flour had better emulsifying activity index than whole flour at all the pH and salt concentrations considered. The increase in the concentration of protein increased the surface hydrophobic constituents of protein which correlated with an increasing index of emulsifying activity and decreasing interfacial tension (Sikorski, 2002).

The effects of pH and salt concentrations on emulsifying activity index (ESI) of ADF and AWF are presented in Figures 5 (a, b and c). At all salt concentrations, the ESI of the tested samples decreased from pH 2-4 and thereafter increased as the pH increased from 6-10. The lowest ESI at 0.0, 0.5 and 1.0 M NaCl concentrations for ADF and AWF were obtained around their isoelectric pH (pH 4) and the maximum values at pH 10. The result showed that addition of salt decreased the ESI of the samples. The trend was similar to the observation of Osman *et al.* (2005) who stated that addition of NaCl significantly decreased the ESI of untreated chickpea flour. The low ESI at low pH and high salt concentration may be attributed to increased interactions between the emulsified droplets, as net charge on the protein is decreased by the presence of chloride ions and increase in emulsion capacity with increase in pH suggests that droplet size decreases with increase in pH beyond the isoelectric point as reported earlier by Chavan *et al.* (2001). The result showed that addition of salt decreased the ESI of the samples significantly. The trend was similar to the observation of Osman *et al.* (2005) who stated that addition of NaCl significantly decreased the ESI of untreated chickpea flour. With respect to NaCl concentration, the highest ESI were observed at 0.0 M compared with 0.5M and 1.00 M NaCl concentrations. The studies of Chavan *et al.* (2001) revealed also that as pH increases, the coulombic repulsion increases between neighbouring droplets, coupled with increased hydration of the charged protein molecules which results into reduction of interfacial energy and combination of emulsion droplet. This may have accounted for the high ESI observe in the studied samples at 0.00 M. Sample ADF had higher ESI than AWF. Osman *et al.* (2005) reported that the stabilizing effect of proteins in emulsions results from the protective barrier they form around fat droplets, which further prevents their coalescence. This might be the reason for better emulsifying properties of defatted flour (ADF) than the full fat flour (AWF).

CONCLUSION

This study has shown that the ackee apple being an under-utilized fruit has functional properties comparable with other known plant protein sources. The defatted flour of the arils of the ackee apple has been shown to exhibit better functional properties than the full fat flour. However, both the defatted and the full fat flours of ackee apple arils have potential to be used as food ingredient.

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