SOME PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF *KARIYA (HILDEGARDIA BATERII)* KERNEL FLOURS

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ABSTRACT

Some physico-chemical and functional properties of *kariya* kernel flours were investigated with a view to exploiting its potentials as ingredients in food applications. Proximate composition showed that protein and crude fat were: 28.68 and 40.37 g/100 g for the full fat flour, and 39.20 and 3.75 g/100 g for the defatted flour respectively. Nitrogen solubility, water absorption capacity, oil absorption capacity, *in-vitro* protein digestibility, foaming capacity, foaming stability, emulsion capacity, emulsion stability and bulk density of the defatted flour were: 72.36, 124.37, 106.45, 79.36, 40.00, 33.94, 29.68, 336.03% and 573.05 kg/m^3, respectively. The water and oil absorption capacities, foaming properties and emulsion properties of defatted *kariya* flour were found to be affected by NaCl concentration and pH. The least nitrogen solubility of defatted *kariya* flour occurred at pH 4, hence the iso-electric pH of the protein. In view of the good functionality of *kariya* kernel flours, it may be explored as a potential source of vegetable protein especially in the tropics where the tree has find favourable conditions for growth.

Keywords: Physico-Chemical, Functional Properties, *Kariya* Kernels, Protein

INTRODUCTION

Until recently, soy protein has been the main oilseed protein commonly used as a functional ingredient in foods. In view of the urgent need for alternative protein sources in the poor countries of the world, screening efforts for novel sources of concentrated proteins and the development of appropriate technologies to optimize their utilization have become necessary (Apata and Ologhobo, 1994; Ezeagwu and Gowda, 2006; Owusu-Ansah and McCurdy, 1991). Several reports indicate that quite a number of unconventional food resources are high in nutrients and can relieve critical food shortages if given adequate promotion and research attention (Mohan and Janardhanan, 1994; Ezeagu *et al*., 2004). In this regard, exploitation of some wild legumes and oilseeds has proven to be viable alternatives for expanding protein sources.

Kariya is an ornamental tropical tree which produces an underutilized edible oilseed. In some West Africa countries, raw or roasted *kariya* kernels (Fig. 1) are eaten like peanuts or used as traditional food condiments. In its fruiting season, one *kariya* tree produces numerous one seeded pods, which often times end up in the garbage; whereas it has been reported that *kariya* kernel is a potential source of protein and fat (Inglett, 1973; Ogunsina *et al.,* 2010). The development of new proteins from such unpopular species requires a pool of information regarding their functional, physicochemical, nutritional and toxicological properties for optimal utilization and consumer acceptance (Alobo, 2004). Many important functional properties concerning water-protein interactions (solubility, foaming, gelation and emulsification) play important roles in the sensory characteristics of foods and their physical behaviour as ingredients in food systems (Adebowale and Lawal, 2004). Various food industries capitalize on the desirable functional properties and improve them to meet specific requirements. However, the dearth of information regarding the physico-chemical and

Fig. 1. Samples of dry *kariya* (*Hildegardia baterii)* nuts and kernels

Functional properties of *kariya* seeds have placed great limitations on its exploitation as an ingredient in food applications. Following previous work on the engineering and nutritional properties of *kariya* seeds (Ogunsina *et al.,* 2012), this study investigates the physico-chemical and functional properties of *kariya* kernel flour with a view to providing useful information for its possible exploitation in food system.

MATERIALS AND METHODS

Source of Materials and Sample Preparation

Matured and dry *kariya* pods were obtained from ornamental trees in Obafemi Awolowo University Staff quarters, Ile Ife, Nigeria between January and March, 2012. Nuts were extracted from the dry pods, shelled and cleaned to remove all extraneous materials. The kernels were stored in air-tight containers under refrigeration until the time of use.

Defatted flour was prepared using the method of Gbadamosi *et al.* (2012). The nuts were shelled to obtain kernels, which were comminuted using Marlex Excella grinder (Marlex Appliances PVT., Daman) and sieved to pass through a standard 150 μm sieve. The flour obtained was divided into two equal portions; one portion was retained as full fat *kariya* flour (FFKF) and the other was defatted by four repeated washing with acetone until the fat content was about 4%. The defatted sample was air dried at 28±2°C for about 24 h (Arogundade *et al*., 2004) to remove residual solvent and was designated as defatted *kariya* kernel ?our (DKKF). The two ?our samples (FFKF and DKKF) were stored in airtight plastic containers and kept refrigerated until the time of use.

Proximate Analysis and in-vitro Protein Digestibility

The flour samples (FFKF and DKKF) were analysed for crude protein, fat, ash, crude fibre and moisture content according to AOAC methods (AOAC, 2002). Carbohydrate was estimated by weight difference. Energy content was derived using Artwater factors in which energy in kCal / 100 g of sample = 9fat + 4protein + 4carbohydrate (Alobo *et al*., 2009; Ogunsina *et al.,* 2010a). Values obtained were averages of triplicates.

In-vitro digestibility (IVD) of proteins was determined using pepsin-pancreatin enzyme systems according to the modified method of Saunder *et al.* (1973) (Gbadamosi *et al*., 2012). A 250 mg sample was suspended in 15 mL of 0.10 M HCl containing 1.5 mg of pepsin, and shaken gently for 1 h at 37°C. The resultant solution was neutralised with 0.50 M NaOH and treated with 4 mg pancreatin (from porcine pancreas, activity equivalent to 4×US Pharmacopeia) in 7.5 mL of phosphate buffer (0.10 M, pH 8.0). The mixture was shaken for 24 h at 37°C inside water bath. The undigested solids were separated by centrifugation, washed with distilled water, air dried and extracted with 0.1 N NaOH. Afterwards, the soluble protein was estimated and IVD was calculated according to equation (1) (Markwell *et al*., 1978).

$$\text{IVD}, \% = \frac{I - F}{I} \times 100 \qquad (1)$$

where, I = protein content of DKKF before digestion, and F = protein content of DKKF after digestion.

Functional Properties

Water and oil absorption capacities (WAC and OAC) were determined following the method of Sathe *et al.* (1982). About 1.0 g of the defatted flour was mixed with 10 ml distilled water (or refined groundnut oil for OAC) for 30 s in a 15 ml centrifuge tube. The mixture was centrifuged at 4000 g for 30 min and volume of supernatant was measured using a graduated measuring cylinder. The value of WAC or OAC was expressed as grams of water or oil bound per 100 g of flour; taking the density of water and that of groundnut oil as 1 and 0.9 g/mL, respectively. The effect of pH (2 - 10) and NaCl concentration (0 1.0 M) on WAC was investigated. Adjustment of pH was carried out with 0.1 M HCl or 0.1 M NaOH using a pH meter.

Nitrogen solubility of the sample was investigated according to the method of Chobert *et al.* (1988) considering pH ranges 2 - 10. Samples were dispersed in distilled water (1% w/v) and mixed thoroughly at the room temperature for 5 min using a magnetic stirrer. After 45 min of stirring at room temperature, the pH was measured (or readjusted if necessary) and the samples were centrifuged at 7,000 g for 30 min. The supernatant was ?ltered to obtain a clear solution and the nitrogen content in the supernatant was determined by Kjeldahl method (AOAC, 2000). There were triplicate determinations and nitrogen solubility was determined using equation 2.

$$\text{Nitrogen Solubility, \%} = \frac{N_S}{N_T} \times 100 \qquad (2)$$

where, N_S = Nitrogen content of the supernatant; N_T = Total nitrogen content of the sample.

The profile was obtained by plotting average values of percent nitrogen solubility against pH.

Least gelation concentration (LGC) was determined using the method of Coffman and Garcia (1977) as documented by Alobo (2004). Sample suspensions of 2 20% (w/v) at 2% interval were prepared in distilled water. The suspensions were transferred into test tubes and heated in a boiling water-bath for 1 h and cooled. The sample in tube was further cooled for 2 h at 4°C and the LGC was taken as the concentration at which the test tube content did not fall or slip when inverted.

Foaming capacity was determined according to Coffman and Garcia (1977) method. About 2 g of the defatted flour was dispersed in 100 ml distilled water and whipped vigorously for 2 min in a kitchen blender at speed 1. The volumes were recorded before and after whipping and the percentage increase in volume was calculated using equation (3).

$$\text{\% increase in vol} = \frac{V_2 - V_1}{V_1} \times 100 \qquad (3)$$

where, V_1 = volume of solution before whipping, and V_2 = volume of solution after whipping.

Foam stability was determined as the volume of foam that remains after 8 h at room temperature (30±2°C) and expressed as the percentage of initial foam volume. The effect of pH (2-10) and ionic strength (0-1 M) using NaCl on the foaming properties were then studied.

Bulk density of the flour samples was determined by putting 50 g into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume and bulk density was calculated as a ratio of the weight of sample to the volume of the sample after tapping.

The effect of pH and salt concentration on emulsifying activity index (EAI) of FFKF and DKKF was studied following the procedure of Wanasundara and Shahidi (1997) as modified by Gbadamosi *et al.* (2012). The protein sample (500 mg) each was dispersed in 100 ml of 0.2 - 1.0 M NaCl. Similarly, 500 mg each of the samples was dispersed in 80 ml of distilled water and the pH of the mixture was adjusted to range between 2-10 with either 0.1N NaOH or HCl to investigate the effect of pH on emulsifying properties. The resulting mixture was then made up to 100 ml with distilled water while keeping the pH constant. The protein solution was mixed with 50 ml of refined groundnut oil and then homogenised using a

kitchen blender at the maximum speed for 60 s. Fifty microlitres aliquot of the emulsion was transferred and mixed with 5 mL of 0.1% (w/v) sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using Spectronic 20D spectrophotometer (Bran Scientific and Instrument Co., England). The emulsions were allowed to stand for 10 min at room temperature and emulsion stability and emulsion activity indices (ESI and EAI) were determined using the expressions (equations 4 & 5) of Aluko and Yada, 1993; Pearce and Kinsella, 1978 respectively.

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{protein weight}} \quad (4)$$

$$ESI\ (min) = \frac{A_{10} \times \Delta_t}{\Delta A} \quad (5)$$

where, A_0 = absorbance at 0 min after homogenisation, A_{10} = absorbance at 10 min after homogenisation; $\Delta t = 10$ min; and $\Delta A = A_0 - A_{10}$.

RESULTS AND DISCUSSION

Proximate Compositions of Full Fat and Defatted Kariya Kernel Flour

The proximate composition of the full fat and defatted *kariya* kernel flours are shown in Table 1. The crude protein and crude fat contents of FFKF were 28.68 and 40.37%, respectively. Upon defatting, the protein content of the full fat flour increased to 39.20% while crude fat reduced to 3.34%. This compares favourably with 38.8% of crude protein earlier reported by Inglett *et al.* (1973) for defatted *kariya* flour.

The protein content of FFKF in this study compared with 25.3% for groundnut, 26.2% for fenugreek and 23.9% for nigerseed (Gopalan *et al.*, 2007). Whereas, the crude fat content compared with those of moringa, 36.18% (Ogunsina *et al.,* 2010a); sponge gourd seeds, 39.1 % (Ogunsina *et al.,* 2010b); groundnut, 39.8%; linseed, 37.1%; mustard seed, 39.7% and niger seed, 39% (Gopalan *et al.,* 2007). The defatted flour exhibited increase in carbohydrate and ash. Similar findings have been documented for soyabeans, beniseed, cashew nut and moringa kernel flours (Egbekun and Ehieze, 1997; Alobo *et al.,* 2009 and Ogunsina *et al.,* 2010a). The high protein contents of both FFKF and DKKF suggest that the flours could be used to fortify or supplement cereal and tuber flours which are very low in protein. The ash content of defatted flour (6.40%) suggests that the defatted flour could be a good source of both macro and micro mineral elements. However, it is needful to note that investigation of the products for trypsin inhibitors, phytate, tannins, saponins and other anti-nutritional factors may provide valuable information regarding the extent and scope of its utilization in food systems.

Table 1. Proximate Composition of Full Fat and Defatted *Kariya* Kernel Flours

Proximate Component (g/100 g)	Full fat *kariya* flour	Defatted *kariya* flour
Ash	4.40±0.15[a]	6.72±0.11[b]
Crude Fat	40.37±0.22[a]	3.34±0.31[b]
Protein	28.68±0.18[a]	39.20±0.62[b]
Crude Fibre	3.39±0.24[a]	1.81±0.08[b]
Carbohydrate	23.15±0.33[a]	48.93±0.12[b]
Energy (kCal/100 g)	570.65	382.59

In vitro Protein Digestibility

In vitro protein digestibility of DKKF was 72.36% which was significantly higher than the 52.28% obtained for conophor defatted flour. (Gbadamosi *et al.*, 2012). The values compared favourably with those of conophor protein isolates (73.47%) and soybean meal (76.08%). The high *in vitro* digestibility value of DKKF might be due to loss of some anti-nutrients like non-polar tannin. The result suggests that defatted *kariya* kernel flour could be explored in food formulation.

Gelation Properties

The least-gelation concentration (w/v) of the defatted flour is shown in Table 2. It shows that the flour exhibits partial gelation at 14% and full gelation from 16% and above. At concentration below 14%, no gelation was observed. The ability of proteins to form gels

Table 2. Least Gelation Concentration of Defatted *Kariya* Kernel Flour

S/N	Least Gelation Concentration (%)									
	2	4	6	8	10	12	14	16	18	20
1	-	-	-	-	-	-	±	+	+	+
2	-	-	-	-	-	-	±	+	+	+
3	-	-	-	-	-	-	±	+	+	+
4	-	-	-	-	-	-	±	+	+	+

varies for different oilseeds and legume flours (Moure *et al*., 2006). Sathe *et al.* (1982) suggested that this variation depends on the relative ratios of different constituents (proteins, carbohydrates and lipids), and the interactions between such components. According to Wu *et al.* (2009), protein gels are composed of three dimensional matrices or intertwined networks, partially associated with polypeptides in which de-ionized water was entrapped. Protein gelation is vital in the preparation and acceptability of many foods, including vegetable and other products; it plays a major role in the preparation of many food proteins. The appearance of gel and gelation mechanisms is fundamentally controlled by the balance between attractive hydrophobic interactions and repulsive electrostatic interactions. The repulsive forces are due to surface charges and the attractive forces are due to various functional groups exposed by the thermal unfolding of the protein (Akintayo and Oshodi, 1999).

Water and Oil Absorption Capacity

At the natural pH of the DFKF sample in water, the WAC was 124.37% while OAC was 106.45% (Table 3). The WAC of DFKF was significantly lower than those of conophor defatted flour (412%) and prickly pear seed flour (316%) (Gbadamosi *et al*., 2012; Nassar, 2008). It was higher than the value (81%) reported for defatted cashew nut powder (Ogunwolu *et al*., 2009). A number of factors including size, shape, hydrophilic-hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins affect the ability of proteins to bind water (Adebowale and Lawal, 2004). *Kariya* seed proteins may find applications when incorporated into aqueous food formulations, especially those involving dough handling. The OAC of DFKF was low compared to those of defatted flours of conophor (257.7%) and cashew kernel (205%) (Gbadamosi *et al.*, 2012; Ogunwolu *et al.,* 2009). Fat absorption is an important property in food formulations because fat improves food flavour and palatability (Odoemelam, 2003), and it determines whether the protein materials will perform well as meat extenders or analogs (Alobo *et al.,* 2009; Ogunsina *et al.,* 2009).

Table 3. Functional Properties Of Defatted *Kariya* Kernel Flour

Functional properties	Defatted flour
Water absorption capacity (%)	124.37± 3.93
Oil absorption capacity (%)	106.45± 0.95
In vitro protein digestibility (%)	72.36 ± 0.38
Foaming capacity (%)	40.00 ± 0.91
Foaming stability (%)	33.94 ± 0.53
Emulsion capacity (m^2/g)	68.29± 0.037
Emulsion stability (min)	53.57± 18.69
Bulk density (kg/m^3)	573.05± 9.82

Bulk Density

Table 3 summarizes the functional properties of DKKF. The tap bulk density of the defatted flour (573.05 kg/m^3) compared favourably with those of soybeans (560 kg/m^3) (Padilla *et al*., 1996) and cashew kernels (710 kg/m^3) (Alobo *et al.,* 2009); but higher than the value of 380 kg/m^3 reported for moringa kernels (Ogunsina *et al*., 2010a). Padmashree *et al.* (1987) reported that higher bulk density is desirable, since it helps to reduce the paste thickness which is an important factor in convalescent and child feeding. Bulk density is an important factor in food products handling, packaging, storage, processing and distribution. It is particularly useful in the specification of products derived from size reduction or drying processes (Mohsenin, 1986).

Effect of pH on Nitrogen Solubility of Defatted Kariya Kernel Flour

The nitrogen solubility profile of the DKKF is shown in Fig. 2. The flour exhibited minimum solubility at pH 4, indicating that the isoelectric point of proteins of DKKF may likely be around pH 4. According to Sorgentini and Wagner (2002), the occurrence of minimum solubility near the isoelectric point is due primarily to the net charge of peptides, which increases as pH moves away from the isoelectric point; and surface hydrophobicity, which promotes protein aggregation and precipitation. Moving away from the pH of minimum solubility, nitrogen solubility increased on either side. Alkaline pH was however more effective in solubilizing DKKF proteins compared to acidic pH. The results of this study compared favourably with those of conophor defatted flour (Gbadamosi *et al*., 2012) and gingerbread plum flour (Amza *et al.,* 2011).

Effect of pH and Salt Concentration on Water Absorption Capacity

The effects of NaCl concentration and pH (2-10) on WAC of DKKF are presented in Figs. 3 and 4, respectively Water absorption capacity of the flour increased with increase in ionic strength of the solution up to 0.6 M. Further increase in ionic strength from 0.8 to 1.0 M led to progressive decrease in WAC of the flour. Also, as the pH increased from 2 to 4, the WAC increased from 103.90 to 132.63%. Further increase in pH led to decrease in WAC of the flour, which implied that the WAC of the flour depends on pH and ionic strength. Increase in ionic strength enhanced protein unfolding and exposed the buried functional groups, and these enhanced WAC. At higher salt concentrations, much of the water was bound to the salt ions, causing dehydration and subsequent reduction in WAC. Although proteins exhibit minimal properties at isoelectric pH, the high WAC at pH 4 may be associated with the

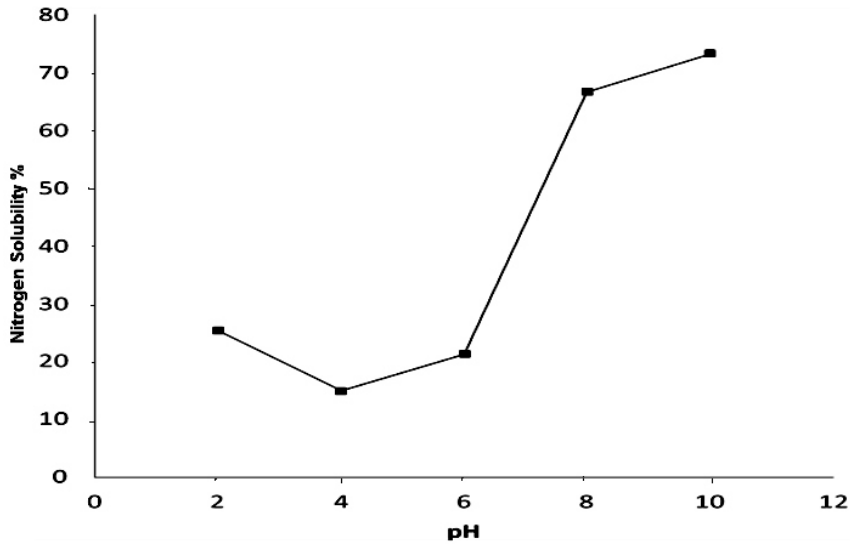

Fig. 2.Effect of pH on Nitrogen Solubility of Defatted Kariya Kernel Flour

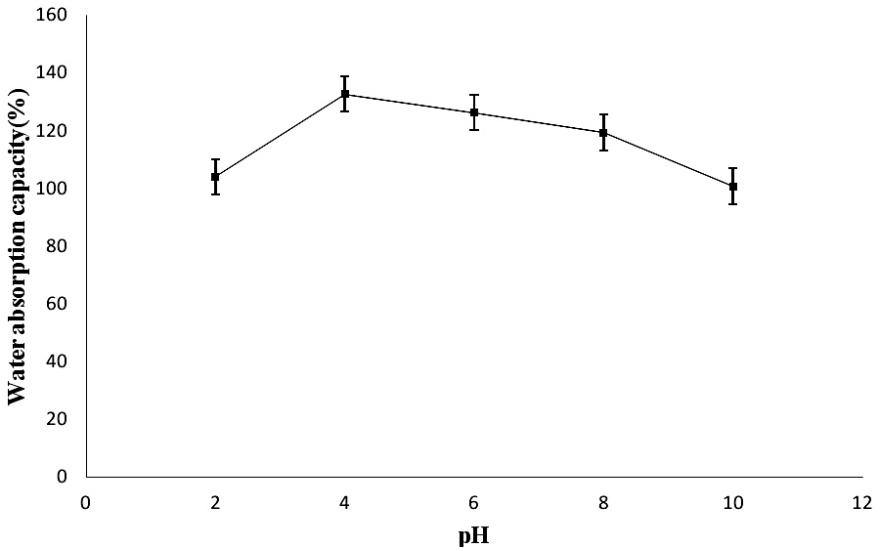

Fig. 3. Effect of pH on Water Absorption Capacity of Defatted *Kariya* Kernel Flour

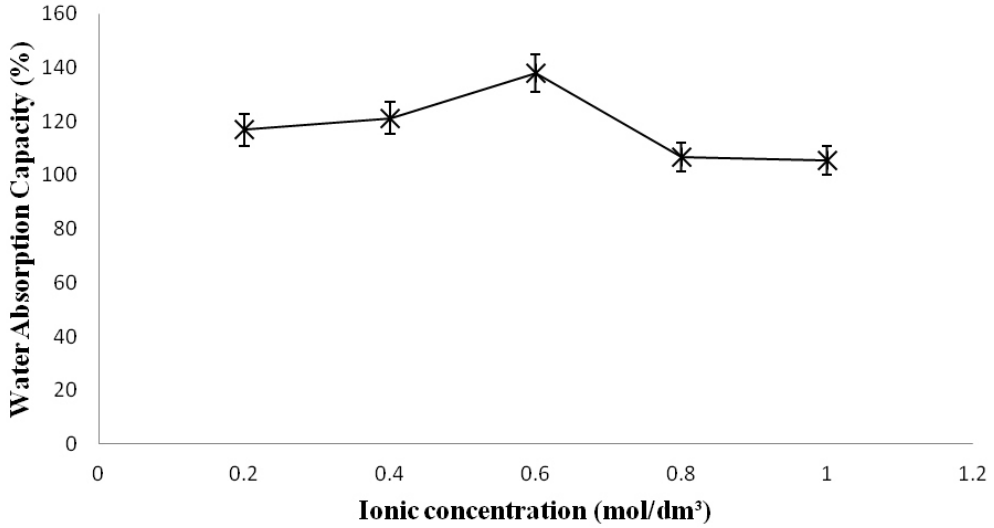

Fig. 4.Effect of Nacl on Water Absorption Capacity of Defatted *Kariya* Kernel Flour

presence of carbohydrates and other non-protein materials in the sample. In functional foods such as sausages, custards and doughs, proteins imbibe water, but do not dissolve because of insufficient water; they therefore get swollen and viscous. These suggest that DKKF could be employed as an ingredient in the preparation of comminuted food products. These properties enable bakers to add more water to doughs for improved handling and freshness in the product.

Effects of pH and Salt Concentration on Emulsion Properties of Defatted Kariya Kernel Flour

The effects of pH and ionic strength on emulsion activity and stability indices (EAI and ESI) are shown in Figs. 5 and 6 respectively. The maximum EAI and ESI were observed at pH 10, while minimum values were recorded at pH 4. The EAI and ESI have been reported to be influenced by pH for proteins of *Cajanus cajan*, *Vigna unguiculata*, *Phaseolus lunatus*, *Canavalia ensiformis, Phelsuma angularis, Pedilanthus calcaratus, Dolichus lablab* (Mwasaru *et al.,* 1999; Chel-Guerrero *et al*., 2002; Chau and Cheung, 1998). At the isoelectric pH, proteins generally exhibit minimal properties owing to the minimal net charge at this point. The net charge at the hydrophilic-lipophilic interface is dependent on pH of the solution and it may impede or facilitate EAI of the protein. The results agreed with the general correlation between emulsion properties and protein solubility as earlier reported (Abbey and Ibeh, 1988; Shanmujasundaram and Venkataraman,

1989). Ogunwolu *et al.* (2009) reported improved ESI at the region of iso-electric pH and this agrees with the observations for cashew and conophor proteins (Ogunwolu *et al.*, 2009; Gbadamosi *et al.*, 2012). The relatively high ESI observed at pH 10 and 2 may be as a result of higher levels of solubilised proteins, which on the other hand may influence ESI through film encapsulation and a balance of the attractive van der Waals and repulsive electrostatic forces (Chau and Cheung, 1998).

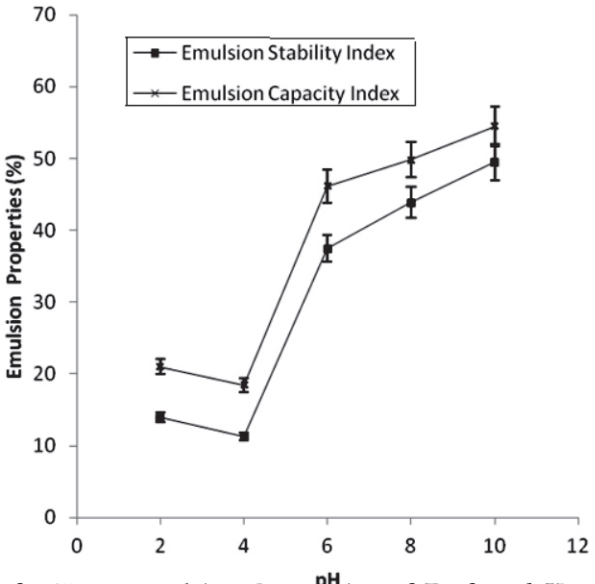

Fig. 5. Effect of pH on Emulsion Properties of Defatted *Kariya* Kernel Flour

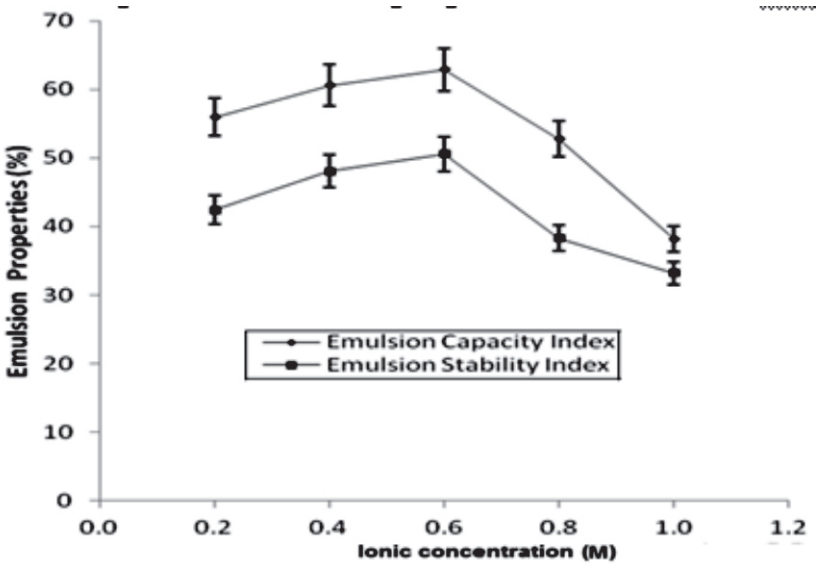

Fig. 6. Effect of Nacl on Emulsion Properties of Defatted *Kariya* Kernel Flour

Emulsifying properties of DKKF increased progressively as ionic strength increased until it reached the highest value in 0.6 M NaCl solution; afterwards, a decline was observed until the minimum was attained in 1.0 M NaCl concentration. The effect of ionic strength on the emulsifying properties of cowpea protein had earlier been reported (Aluko and Yada, 1995). Chavan *et al.* (2001) also reported ionic strength dependent emulsifying properties for beach pea. Wagner and Gueguen (1995) attributed higher ESI of soy protein at low ionic strength to dissociation of oligomeric structure of 11S-glycinin and subsequent improvement of surface behaviour.

Emulsifying properties of proteins depend basically on substantial decrease in interfacial energy due to the adsorption of the protein at the oilwater interface and the

electrostatic, structural and mechanical energy barrier caused by the interfacial layer that opposes destabilisation processes (Wagner and Gueguen, 1999). Initial increase in ionic strength of the solutions up to 0.6 M enhanced the formation of charged layers around the fat globules and this resulted in mutual repulsion among them. Also at low ionic strength, formation of hydrated layer around the interfacial material resulted in lower interfacial energy and retarded droplet coalescence. At higher ionic strength (6.0-1.0 M), protein unfolding was decreased. This development probably limited adsorption of the protein at the oilwater interface.

Effects of pH and Salt Concentration on Foaming Properties of Defatted Kariya Kernel Flour

Effects of pH and ionic strength using NaCl on foam properties were presented in Figs. 7 and 8, respectively. The foam capacities (FC) and foaming stability (FS) of the defatted flour followed similar pattern. Ogunsina *et al.* (2010a) and Alobo *et al.* (2009) gave similar account on defatted moringa and cashew flours, respectively. The removal of fat and denaturation of proteins due to changes in pH could expose more solubilised proteins, which in turn, can increase FC and FS (Sathe and Salunkhe, 1981; Narayana and Narasinga-Rao, 1982). From pH 2 to pH 4 FC decreased and from pH 4 to 10, it increased. Generally, it was observed that FC increased with increase in pH after the iso-electric point. The effect of ionic strength using NaCl on FC and FS of the flour followed similar pattern. It was observed that foaming increased as NaCl concentration increased until it reached 0.6 M; and afterwards there was a decline. The highest value for FC and FS were 32% and 29% at 0.6 M NaCl concentration respectively. Similar patterns were reported for cashew (Alobo *et al.,* 2009). Akintayo *et al.,* (1999) gave an account of the influence of ionic strength on foaming properties of pigeon pea protein concentrates and showed that increase in ionic strength up to 0.5 M improved the FC, while further increase in ionic strength to 1.0 M reduced FC. The results showed that both FC and FS were pH and ionic strength using NaCl dependent.

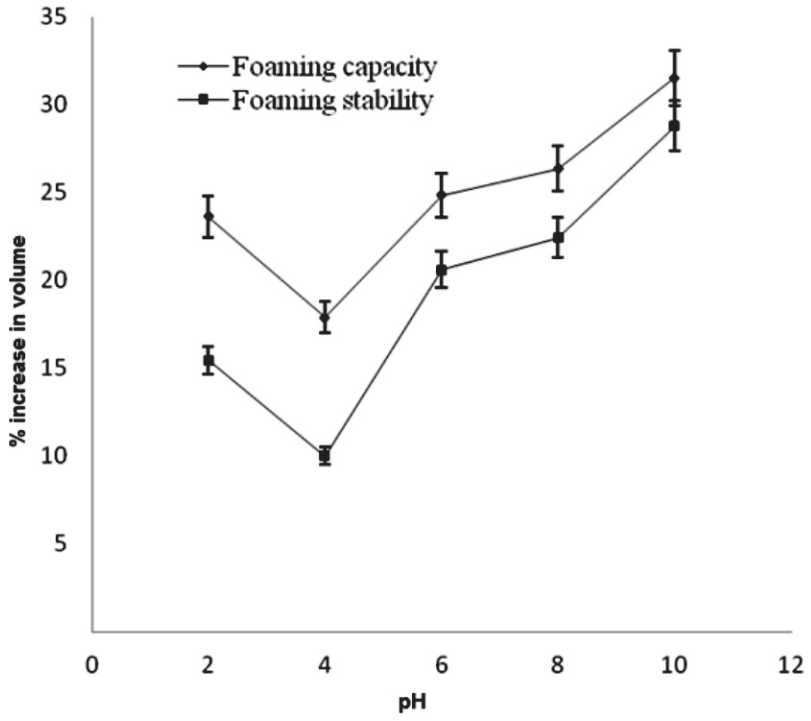

Fig. 7. Effect of pH on Foaming Properties of Defatted *Kariya* Flour

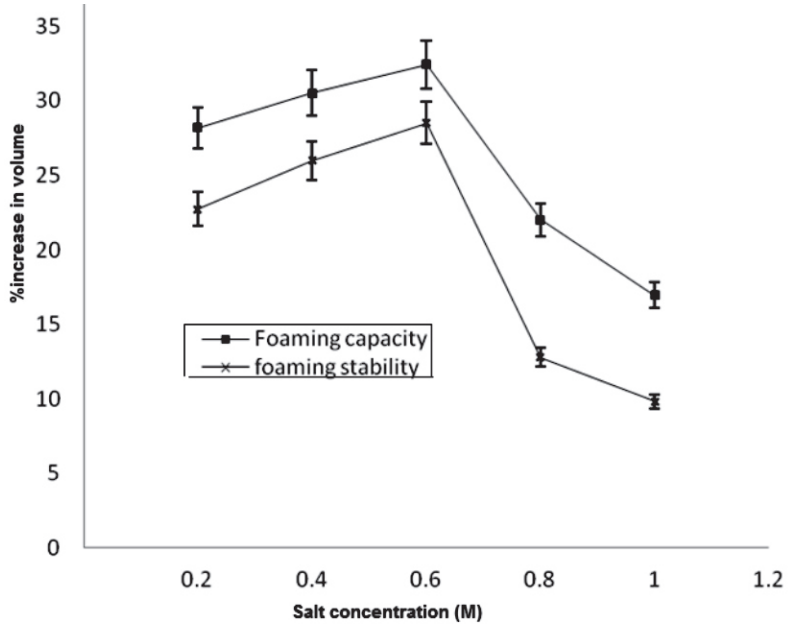

Fig. 8. Effect of Nacl on Foaming Properties of Defatted *Kariya* Kernel Flour

CONCLUSIONS

Kariya kernel flour was found to be a good source of oil and protein. The oil, if well harnessed could present opportunities for food and industrial uses. The high *in vitro* protein digestibility, relatively high water and oil absorption capacities, excellent gelling capacity and emulsifying properties of DKKF could make it suitable as protein foods and as functional ingredients. This is suitable in the tropics where there is poor protein nutrition and *kariya* tree has found favourable conditions for growth.

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