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PHYSICO-CHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED KARIYA (HILDERGARDIA BARTERI) SEED FLOUR

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ABSTRACT

This study was undertaken to evaluate the effect of fermentation on the functional, antioxidant properties and *in-vitro* protein digestibility of Kariya seed flour. Kariya seeds were fermented by transferring the seeds in calabash pots lined with banana leaves. Fermented products were taken out after 96 h to determine functional properties, *in-vitro* protein digestibility and antioxidant properties. The water absorption capacitiy (WAC) was significantly ($p < 0.05$) higher in raw fermented kariya (RFK) and raw unfermented kariya (RUK) (137.47%, 136.90% respectively) than in cooked unfermented kariya (CUK) and cooked fermented kariya (CFK) (122.50%, 106.63% respectively). The bulk densities were significantly ($p \leq 0.05$) higher in RUK and CUK (0.59 g/ml, 0.59 g/ml) than in RFK and CFK (0.53 g/ml and 0.54 g/ml). Fermentation and cooking significantly ($p<0.05$) increased *in-vitro* protein digestibility of the samples (63.71% – 85.51%). There was an increase in the free radical scavenging activity with respect to fermentation with sample RFK having the highest radical scavenging activity value (88.59% at 5mg/ml) followed by CUK and CFK (83.70% and 78.40% respectively at 5mg/ml), while the lowest DPPH radical scavenging activity was obtained with RUK (71.12%) sample at the same concentration. Kariya flour could serve as a possible source of antioxidants for use in food.

Keywords: *Water absorption capacity, bulk density, least gelation, in-vitro protein digestibility and antioxidant property*

1. INTRODUCTION

A number of oil seeds have been characterised but the vast majority have not been adequately evaluated. This is also particularly valid for the Nigerian flora which has one of the most extensive floras in the continent of Africa (Oderinde and Ajayi, 1998). There has been a focus on non-conventional oil seeds for possible development and use (Obasi and Okolie, 1993). *Hildegardia barteri* (Kariya) falls into this group of underutilized species of plants.

Kariya (*Hildegardia barteri)* is primarily an ornamental tree in West Africa that grows from the Ivory Coast to Nigeria and is called the Krobo Christmas tree (Irvine, 1961), grown only for its bright beautiful flowers which blossom during the dry season. *H. barteri* is a tree that has distinct ornamental value since its flowers are conspicuous on leafless branches in the dry season. The mature pods drop completely when dry and are disposed as refuse in many places. The seeds of *Hildegardia barteri* are consumed in West Africa as raw or roasted nuts and have a flavour resembling peanuts and sometimes used as condiments in traditional food preparations. The proximate analysis showed that kariya kernels contain 17.5, 37.5, 2.8 and 6.5% of crude protein, crude fat, ash and crude fibre, respectively (Ogunsina *et al*., 2011).

Research efforts on kariya have been limited to the composition of the seed (Inglett *et al*., 1973), nutritional and physical properties of Kariya seed (Ogunsina *et al*., 2011), physical, functional and nutritional properties of kariya seeds defatted flours by Adebayo *et al.* (2013), and also on the physicochemical characteristics of *Hildegardia barteri* seed oils obtained by cold pressing and solvent extraction procedures by Adebayo *et al*. 2015. Furthermore, kariya seeds have found limited application as foods and food ingredient. To increase its utilization, there is need to process the whole flour into defatted flours and then examine the suitability of these defatted flours as functional ingredients and food supplements. However, the ultimate success of utilizing any plant protein as food ingredients largely depends on its functional and nutritional properties.

As a result of increasing demand for conventional protein seeds such as soybean, peanut, rape seed and canola, there is need to source protein from underutilized oilseeds. Fermentation has over the years been demonstrated to enhance the biological enrichment of food substrates with protein, essential amino acids, vitamins, and elimination of anti-nutrients (McGovern *et al*., 2004). Fermented foods have further benefits of providing bio-nutrients and minerals and enhancement of flavour and aroma. The process also increases digestibility and exert health promoting benefits (Jeyaram *et al*., 2009). Fermentation has also been reported to improve antioxidant properties of soybean meal using *Lactobacillus plantarum* Lp6 (Amadou *et al*., 2009).

Antioxidants are substances that are added to foods to slow the rate of lipid oxidation reaction and therefore maintain food freshness. It inhibits the formation of free radicals and hence contributes to the stabilization of the lipid sample. Antioxidants are said to play significant role in the body defense system against

Reactive Oxygen Species (ROS) (Vivek *et al*., 2006) that inhibit oxidative mechanisms that lead to degenerative diseases such as artherosclerosis, cardiovascular diseases and cancer. Recently, the use of synthetic antioxidants has been implicated and efforts are now on at finding alternatives from natural sources. Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention (El Diwani *et al*., 2009). Oil seeds (soybean, peanut, rape seed, canola, chickpeas and almonds) generally have also been shown to have antioxidants (Schmidt and Pokorny 2005).

The present study therefore aimed to investigate the influence of fermentation on the physico-chemical composition, antioxidant activities and *in vitro* protein digestibility of kariya seed defatted flour with a view to improving its utilization as food ingredients and a possible source of natural antioxidants.

2. MATERIALS AND METHODS

2.1. Sample Collection and Fermentation

Dried Kariya pods were gathered from ornamental Kariya trees in Obafemi Awolowo University, Ile-Ife. These seeds were sorted manually, to remove stones, damaged and immature seeds. The kernels were divided into four parts. The first part was kept as control (unfermented seeds), the second portion was cooked but not fermented and the third portion was fermented but not cooked. The fourth portion of the kernel was cooked and transferred into a calabash pot, lined uniformly with banana leaves (up to 5 layers) and allowed to undergo natural fermentation at ambient for five days inside the incubator (30 °C). Fermented samples were collected at a-day interval until the end of fermentation period of five days. Fermented and unfermented (control) samples were oven dried in a hot air oven set at 70 °C, grounded using laboratory mill into flour, screened through a standard sieve (200 µm mesh), kept in polythene bags and stored in the freezer until required for analyses.

2.2. Physico-Chemical and Functional Properties

2.2.1. Bulk density

Bulk density was determined by the method of Okezie and Bello (1988). A 10 ml graduated cylinder, previously tared, was gently filled with the sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/ml).

2.2.2. pH measurement

pH was measured by making a 10% w/v suspension of the sample in distilled water. The suspension was mixed thoroughly in a Warring blender (Binatone, BLG-450, China) and the pH was measured with a Hanna checker pH meter (Model HI1270).

2.2.3. Water absorption capacity (WAC)

The WAC was determined at room temperature and at temperatures ranging between 60 to 90°C using a combination of the AACC (1995) method and those of Sosulski (1962) and Rutkowski and Kozlowska (1981). A 2 g sample was dispersed in 20 ml of distilled water. The contents were mixed for 30 s every 5 min using a glass rod and after mixing five times, centrifuged at 1788 × g for 30 min using the centrifuge (0502-1 Hospibrand, USA). The supernatant was carefully decanted and then the contents of the tubes were allowed to drain at a 45° angle for 10 min and then weighed. The water absorption capacity was expressed as percentage increase of the sample weight.

2.2.4. Gelling concentration

The method of Sathe and Salunkhe (1981) was employed for the determination of gelling concentration. Sample suspensions of 1, 3, 5, 7, 9, 11, 13, 15, 17 and 20% (w/v) were prepared in 5 ml distilled water and the test tubes were heated in a boiling water bath for 1 h followed by rapid cooling under running cold tap water. The test tubes were further cooled for 2 h at 4°C. Least gelling concentration was determined as that concentration when the sample from the inverted test tube did not fall down or slip.

2.2.5. In vitro protein digestibility determination

In vitro protein digestibility of samples was measured according to the combined methods of Saunders *et al.* (1973) and as modified by Chavan *et al.* (2001). Two hundred and fifty milligrams of the sample was suspended in 15 ml of 0.1 M HCl containing 1.5 mg pepsin, followed by gentle shaking for 1hr at room temperature. The resultant suspension was neutralized with 0.5 M NaOH and treated with 4.0 mg pancreatin in 7.5 ml of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 24 hr at room temperature. The mixture was then filtered using Whatman No 1 filter paper and the residue washed with distilled water, air-dried and used for protein determination using Kjedhal procedure (AOAC, 2000) as described earlier. Protein digestibility was obtained by using the equation (1);

$$In\ vitro\ protein\ digestibility\ (\%) = \left(\frac{I-F}{I}\right) \times 100 \quad (1)$$

$$where,\ I = protein\ content\ of\ sample\ before\ digestion$$
$$F = protein\ content\ of\ sample\ after\ digestion$$

2.2.6. Protein solubility

The effect of pH on protein solubility was determined by a method described by Gbadamosi *et al*., (2012) with slight modifications. About 2.5 g of the sample was dispersed in 250 ml of 0.1 N NaOH. The mixture was stirred on a magnetic stirrer (Cenco, Netherland) for 1 hr, centrifuged (Harrier 15/80 MSE) at 4,500 g for 30 min and then filtered through Whatman No.1 filter paper. The pH of the filtrate (20 ml) was adjusted to pH 2, 4, 6, 8 and 10 with 1 N HCl or NaOH. The solution was then made up to 30 ml and stirred for another 10 min. An aliquot of the mixture was centrifuged at 12,000 g for 20 min and the protein content was determined using the modified Lowry method (Markwell *et al*., 1978).

2.3. Antioxidant properties of kariya defatted flour

2.3.1. Extraction of antioxidant

Extraction of antioxidants was carried out on samples of defatted flour following the method of Yurttas *et al*. (2000) with minor modifications. Approximately 5g of each of the sample were separately mixed with 200ml of 80% methanol (methanol : water, 80:20 v/v) in a conical flask and the extraction was done on a magnetic stirrer for 8hrs.The mixture was concentrated to dryness on a rotary evaporator and the extract was stored in a refrigerator until use.

2.3.2. DPPH assay

The radical scavenging ability of the extract was determined using the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl

hydrate) as described by (Pownall *et al*., 2010).The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1ml of different concentrations (5, 2.5, 1.25, 0.625, 0.3125 mg/ml) of the extract or standard (vitamin C) in a test tube was added 1ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30mins after which the absorbance was read at 517nm against a DPPH control containing only 1ml methanol in place of the extract.

The percentage of inhibition was calculated as shown in equation (2):

$$Inhibition\ (\%) = \left(\frac{Acontrol - Asample}{Acontrol}\right) \times 100 \qquad (2)$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. . Inhibition concentration leading to 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentrations.

2.3.3. Determination of Ferric Reducing Antioxidant power (FRAP)

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method is based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous coloured form due to the donation of electron by antioxidant compounds.

Procedure: A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5-triazine and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. A 50 ul aliquot of the extract at 1mg/ml and 50 µl of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/ml) were separately added to 1ml of FRAP reagent. The mixture was well mixed and absorbance measurement at 593 nm against reagent blank (50 µl of distilled water and 1 ml of FRAP reagent) after allowing reaction to complete at exactly 10 minutes. The reducing power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard. This was done by plotting the graph of the absorbance of ascorbic acid (standard) against concentration. The equation of the graph obtained was used to calculate the equivalent concentration based on the absorbance obtained for the extracts, and this is expressed as ascorbic acid equivalent per gram of the extract (AAE/g of the extract).

2.3.4. Metal chelating ability assay

The metal-chelating assay was carried out according to the method of Singh and Rajini (2004) with some modifications. Solutions of 2 mM $FeCl_2{\cdot}4H_2O$ and 5 mM ferrozine were separately diluted 20 times. Briefly, an aliquot (1 ml) of diferent concentrations (6.25, 12.5, 25.0, 50.0 and 100.0 mg/ml) of the extracts was mixed with 1ml of diluted $FeCl_2{\cdot}4H_2O$. After 5 min incubation, the reaction was initiated by the addition of 1 ml of diluted ferrozine. The mixture was shaken vigorously and after a further 10 min incubation period the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine–Fe^{+2} complex formations was calculated as shown in equation (3):

$$Metal\ chelating\ activity\ (\%) = \left(\frac{Acontrol - Asample}{Acontrol}\right) \times 100 \qquad (3)$$

Where, $A_{control}$ = absorbance of control sample (the control contains mixture of FeCl2 and ferrozine) and Asample = absorbance of a tested sample.

2.3.5. Determination of total phenol content

The method of determining the total phenolic content was described by Singleton and Rossi, 1965 as described by Gulcin *et al* (2003) using the folin ciocalteu's phenol reagent which is an oxidizing reagent.

Procedure: To a mixture of 0.1ml of sample and 0.9ml of water was added 0.2ml of folin-ciocalteu's phenol reagent and the resulting mixture vortexed. After 5minutes of standing, 1.0ml of 7% (w/w) Na_2CO_3 solution then added and the solution was then distilled to 2.5ml before incubated for 90min at room temperature. The absorbance against a negative control containing 1ml of water in place of the sample was then taken at 750nm. The standard used was the Gallic acid at 0.1mg/ml in order to determine Gallic acid Equivalent (GAE) of sample, after preparing a calibration curve. Distilled water was used as blank.

2.3.6. Determination of total Flavonoid content

Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml was used as standard in comparison to the sample extract. This was carried out based on the aluminium chloride colorimetric assay method as described by Miliauskas *et al.* (2004).

Procedure: To 0.1ml of extract/standard was added 0.4ml of distilled water. This was followed by 0.1ml of 5% sodium nitrite. After 5minutes, 0.1ml of 10% Aluminum Chloride and 0.2ml of sodium hydroxide was added and the volume was made up to 2.5ml with distilled water. The absorbance at 510nm was measured against the blank. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the plant extract is shown in equation (4):

$$X = q * \frac{V}{w} \qquad (4)$$

X = Total content of flavonoid compound in quercetin equivalent

q= concentration of quercetin established from the standard curve

V= volume of extract (ml)

w= weight of the crude methanolic extract obtained

3. RESULTS AND DISCUSSION

3.1. Physico-chemical and functional properties

3.1.1. Bulk density and pH

The bulk density (BD) of fermented and unfermented kariya samples is presented in Table 1. There was no significant difference ($P<0.05$) in the BD of the fermented sample, but were significantly lower than those of the unfermented samples. The values ranged between 0.53 g/ml - 0.59 g/ml. The bulk density values obtained in this study were generally higher than those of defatted cashew nut powder (0.48 g/ml), flour from commercially sold soybean (0.38 g/ml) and fermented *Artocarpus altilis* pulp flour (0.46 g/ml) as reported by Ogunwolu *et al.* (2009), Edema *et al.* (2005) and Appiah *et al.* (2011), respectively. The reduction in bulk density as a result of fermentation is similar to the observation of Appiah *et al.* (2011), Onimawo *et al.* (2003) and Elkhalifa *et al.* (2005) on fermented *Artocarpus altilis*, sorghum flour and pumpkin seed (*Telfaria occidentalis*) respectively, where it was noticed that the fermented samples were less dense than the raw samples. This

decrease in bulk density as a result of fermentation could be attributed to the degradation of high molecular weight compounds by fermenting organisms such as polysaccharides and proteins into simpler substances such as sugars and amino acids and peptides. Bulk density is a measure of heaviness of flour (Adejuyitan *et al*., 2009) and an important parameter that determines the suitability of flours for ease of packaging and transportation of particulate foods (Shittu*et al*., 2005). The decrease in bulk density of fermented flour would be an advantage in the preparation of infant foods. Fermentation has been reported as a useful and traditional method for the preparation of low bulk weaning foods (Desikachar, 1980). Fermentation increased the pH of kariya seeds and the difference was significant in raw fermented kariya sample. The release of ammonia by microorganisms involved in the fermentation may be responsible for the increase (Omafuvbe *et al*. 2000)

3.2. Water absorption capacity

The water absorption capacity (Table 1) ranged from 106.63 % to 137.47 % with RFK exhibiting the highest capacity and CFK the lowest capacity to absorb water. The WAC of the raw samples (RFK and RUK) showed no significant difference but the values were significantly ($P < 0.05$) higher than those of the cooked samples (CUK and CFK). Fermentation had significant ($P<0.05$) effect on the water absorption capacity of the cooked kariya samples. The low WAC exhibited by CUK and CFK might be due to pre-denaturation and pre-gelatinization of proteins and starch respectively during the cooking process. This is also in agreement with the result obtained for cooked milkweed flaked seeds as reported by Hojilla-Evangelista and Evangelista (2009) where it was observed that heating typically reduced WAC in cooked milk weed from 2.91 to 2.72 %. The denaturation and/or aggregation have been reported to reduce the availability of polar amino groups for hydrogen bonding with water molecules (Cheftel *et al*. (1985). The WAC of defatted kariya seed flour was lower than those of conophor defatted flour (412.6 %) and prickly pear seed flour (316 %) as reported by Gbadamosi *et al*. (2012) and Nassar (2008) but compared well with those of fermented tigernut flour (141 %) as reported by Adejuyitan *et al*. (2009), and fermented and non-fermented pigeon pea flour samples (113.0 to 142.0g/100 g reported by Adebowale and Maliki (2011). Hence RFK would be more useful in food system such as baking products which requires hydration to improve handling characteristics.

3.3. Least gelation concentration

As shown in Table 1, there was significant difference ($P<0.05$) in the gelling ability between sample CFK and samples RFK, CUK, RUK which were not significantly different. Sample CFK exhibited the highest least gelation capacity (LGC) (9%) while RFK, CUK, RUK showed the least gelling capacity (5%) and therefore the best gelation capacity. Lawal *et al.* (2007) reported that lower least gelation capacity means better gelation capacity and increase in ionic strength enhance gelation properties. The results showed that combination of cooking and fermentation significantly reduced the ability of kariya flour to form stable gels. This implies that *kariya* flour may be a good gel-forming or firming agent, and could be useful in food systems such as pudding and snacks which require thickening and gelling. Protein gelation is vital in the preparation and acceptability of many foods, including vegetables and other products (Lawal *et al*., 2007).

3.4. *In-vitro* protein digestibility (IVPD)

The in vitro protein digestibility of all the samples is presented in Table 1. Cooking and fermentation were found to cause a significant ($P < 0.05$) improvement in IVPD in all the samples. The increase was from 63.71 to 85.51 in all the samples (RFK, CUK, RUK, CFK, CFK1, CFK2 and CFK3). The cooking process increased the rate at which the anti-nutrients were leached out and protease inhibitors are also destroyed. The improvement in protein digestibility caused by fermentation could be attributed to the partial degradation of complex proteins to more simple and stable products (Chavan *et al*., 1988). It could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes (Hassan, 1995). In addition, the elimination of phytic acid contributes to the improvement in protein digestibility in fermented millet (Khetarpaul, 1988).

Table 1: Functional properties of kariya seed defatted flours at their natural pH

Functional characteristics	RFK	RUK	CUK	CFK
BD (g/ml)	0.53 ± 0.01^a	0.59 ± 0.01^b	0.59 ± 0.01^b	0.54 ± 0.04^a
pH	6.45 ± 0.07^b	6.15 ± 0.07^a	6.05 ±0.07^a	6.20 ± 0.00^a
WAC (%)	137.47 ± 0.08^c	136.90 ± 0.11^c	122.50 ± 0.05^b	106.63 ± 0.01^a
LG (%)	5.00 ± 0.28^a	5.00 ± 0.21^a	5.00 ± 0.57^a	9.00 ± 0.65^b
IVD (%)	82.14 ± 0.04^c	63.71 ± 0.04^a	65.49 ± 0.07^b	85.51 ± 0.03^d

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

RFK – Raw fermented Kariya (96 h); CUK – Cooked unfermented Kariya; RUK – Raw unfermented Kariya; CFK – Cooked fermented Kariya (96 h)

The result obtained in this study is comparable and falls within the values reported by Singh *et al*. (2012) for the IVPD of fermented and unfermented sorghum, pearl millet and maize (65.0 to 83.0, 68.0 to 84.0 and 63.0 to 81.0 %). This also agrees with Mohiedeen *et al.* (2010) who reported that fermentation was found to improve the IVPD of two maize cultivars and this could be attributed to the partial degradation of complex storage proteins into more simple and soluble products. Several workers have observed significant increase of IVPD by natural fermentation. Youssif and El-Tinay (2000) showed that natural fermentation of sorghum increased IVPD from 51.8% to 75.6%. Similarly, Osman, (2004), observed an improvement in protein digestibility during natural fermentation of sorghum for *Khamir* bread preparation. An increase in protein digestibility has also been reported in finger millet and pearl millet when fermented for 24 h (Mbithi-Mwikya *et al*., 2000; Elyas *et al*., 2002). This suggests the possible use of cooked and fermented flour of kariya seed in improving the nutritional qualities of food particularly low protein and protein-deficient foods.

3.5. Protein Solubility

The pH solubility profiles of RFK and RUK are presented in Figure 1. The minimum solubility values of RFK and RUK were found to be 14.19% and 34.07% at pH 4, while the maximum solubility were 47.60% and 77.46% at pH 10 respectively. Kariya proteins was acidic at their isoelectric pH and the solubility was found to rapidly increase below and above their isoelectric point. Sorgentini and Wagner (2002) reported that the occurrence of minimum solubility near the isoelectric point is due primarily to both the net charge peptides, which increases as pH moves away from the isoelectric point, and surface hydrophobicity that promotes the aggregation and precipitation via hydrophobic interaction. It was observed that the alkaline pH range was found to be more effective in solubilising the proteins. The value obtained falls in the range of values of peanut protein isolate (60.05%) and mustard green meal (77.0%) reported by Cherry (1990) and Aluko *et al.* (2005). Since the solubility at pH 10 is

significantly higher than other pH, pH 10 is considered to be the optimum pH to solubilize kariya seed proteins effectively, and according to Idouraine *et al.* (1991) this is an important characteristic for food formulation. Solubility profile over a range of pH values is being used increasingly as a guide to protein functionality, since this relates directly to many important properties, e.g., use in beverages, emulsification, foaming capacity and gelation (Molina *et al.,* 2004).

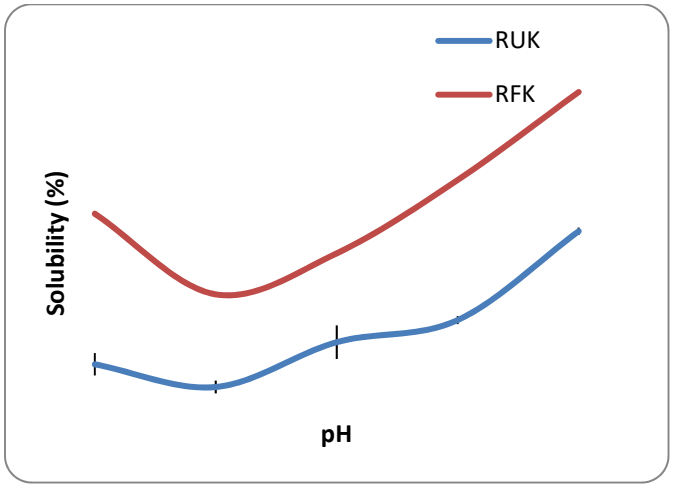

Figure 1. Protein solubility as influenced by pH

RFK – Raw fermented (96 hr) Kariya;
RUK – Raw unfermented Kariya

3.6. Antioxidant properties of kariya seed

3.6.1. DPPH free radical scavenging assay

The DPPH radical scavenging activities of all the extracts were influenced by the concentration as shown in Figure 2. The radical scavenging activities increased significantly ($P < 0.05$) for all the samples. Among the different samples, RFK exhibited the highest radical scavenging activity value (88.59% at 5mg/ml) followed by CUK and CFK (83.70% and 78.40% at 5mg/ml), while the lowest DPPH radical scavenging activity was obtained with RUK (71.12%) sample at the same concentration. Considering the effect of various processing methods on the free radical inhibition activity of seed extract in this study, all the processed samples showed moderate to higher levels of free radical inhibition activity than the raw seeds. The free radical inhibition activities of seed extracts of RFK, CUK and CFK samples were found to be higher than that of the raw seed extract (RUK) at all concentrations. The results obtained in this study agreed with the reports of Asirvatham *et al.* (2011) and Samruan *et al.* (2012) on the antioxidant activity of raw and differently processed underutilized tropical legume (*Canavalia ensiformis*) seeds and antioxidant activity of fermented and raw soybean extract, where it was seen that the cooked and fermented samples were found to have higher antioxidant activity than that of the other samples.

DPPH radical scavenging activity of all the extracts reveal antioxidant potency based on IC_{50} values when compared with ascorbic acid as shown in Table 2. Lower value of IC_{50} indicates a higher antixodiant activity (Kang *et al.*, 2009). Sample RFK and CFK showed higher antioxidant activity than CUK and RUK at IC_{50}values 2.68 mg extract/ml, 2.69 mg extract/ml and 2.82 mg extract/ml 3.37 mg extract/ml. The antioxidant activity of fermented Kariya crude extract showed higher antioxidant activity than the unfermented Kariya crude extract. The result also agrees with that reported by Samruan *et al.* (2012) where fermented soybeans showed higher radical scavenging capabilities. The results showed that the antioxidant activity of fermented and unfermented kariya crude extracts was higher than those of soybean and fermented soybean (IC_{50} values of 21.09 and 14.28 mg extract/ml respectively) as reported by Samruan *et al.* (2012). In conclusion, the high DPPH scavenging properties of RFK and CFK may be due to its high hydrophobicity character than CUK and RUK and this could make it a useful ingredient in preventing oxidative deterioration of foods and hence in disease prevention.

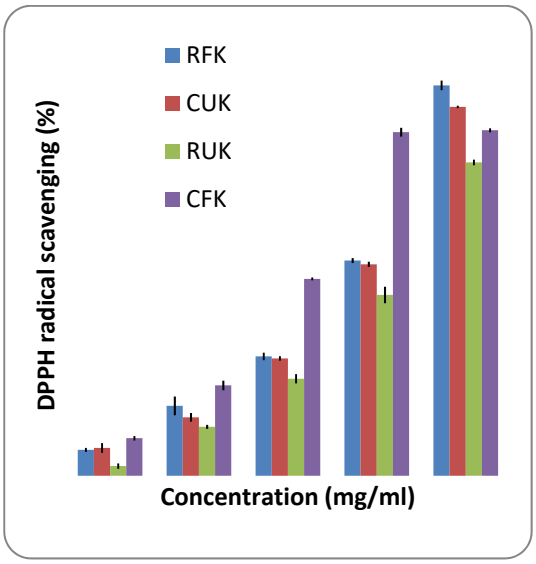

Figure 2: DPPH radical scavenging activities of kariya seed defatted flour at different concentrations

RFK – Raw fermented (96 h) Kariya;
CUK – Cooked unfermented Kariya;
RUK – Raw unfermented Kariya;
CFK – Cooked fermented Kariya (96 h)

3.7. Metal chelating ability assay

The ability of kariya seed extract to chelate and deactivate transition metals is shown in Figure 2. The ferrous ion-chelating abilities of samples CUK and RUK are significantly ($P < 0.05$) greater than that of samples RFK and CFK. As the concentration increased, samples CUK and RUK showed significant transition metal (Fe) chelating ability. Samples RFK and CFK did not have any significant transition metal (Fe) chelating ability irrespective of the days of fermentation compare to the raw seeds. The result of this study correlates with the report of Ibukun (2012), on the antioxidant activities of sesame seed (*Sesanum indicum*), where it was also seen that fermented sesame did not have any significant transition metal (Fe) chelating ability irrespective of the days of fermentation compare to the raw seeds.

In Table 2, the metal chelating (MC) abilities of the extracts revealed antioxidant potency based on the IC_{50} values in comparison with EDTA. At low value of IC_{50} indicates a higher metal chelating activity. The metal chelating activities of CUK and RUK (IC_{50} value at 0.39 and 0.69 mg extract/ml) were higher than RFK and CFK (1.13 and 1.31 mg extract/ml) but both were lower when compared to that of EDTA (0.048 mg extract/ml). The result revealed that samples CUK and RUK had better chelating properties than samples RFK and CFK. The high chelating activity of the extracts could enhance ability of tissues to reduce rate of deteriorative metal-catalyzed lipid oxidation. The extracts may also serve as good agents to prevent metal ion-dependent oxidative damage to food lipids and thereby serve as food preservatives. The

chelating ability is regarded as an antioxidant mechanism to prevent oxidative assault on biological macromolecules such as lipids, proteins and nucleic acids.

3.8. Ferric reducing Antioxidant power (FRAP)

Reducing power is considered a defence mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals (Ogunmoyole *et al*., 2009).

The results of the ferric reducing antioxidant properties (FRAP) presented in Table 2 shows that Ferric reducing properties of samples RFK and CFK were significantly ($p<0.05$) greater than those of samples RUK and CUK.

Table 2: Antioxidant properties of kariya defatted flour

Sample	FRAP (AAE ug/g)	TPC (GAE ug/g)	Flavonoid (QUE ug/g)	DPPH IC_{50} (mg/ml)	MC IC_{50} (mg/ml)
RFK	0.28 ± 0.01^{b}	1.21 ± 0.02^{d}	0.14 ± 0.01^{c}	2.68 ± 0.00^{a}	1.13 ± 0.02^{c}
CUK	0.09 ± 0.00^{a}	0.60 ± 0.01^{b}	0.00 ± 0.00^{a}	2.82 ± 0.02^{b}	0.39 ± 0.01^{a}
RUK	0.26 ± 0.00^{b}	0.47 ± 0.00^{a}	0.03 ± 0.00^{a}	3.37 ± 0.00^{c}	0.69 ± 0.01^{b}
CFK	0.56 ± 0.00^{c}	0.90 ± 0.06^{c}	0.08 ± 0.01^{b}	2.69 ± 0.02^{a}	1.31 ± 0.04^{d}
Ascorbic acid	-	-	-	0.07 ± 0.02	-
EDTA	-	-	-	-	0.04 ± 0.08

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

RFK – Raw fermented Kariya;

CUK – Cooked unfermented Kariya;

RUK – Raw unfermented Kariya;

CFK – Cooked fermented Kariya;

EDTA: Ethylene diamine tetra-acetate;

TPC: Total phenol content;

DPPH: (diphenyl-1-picryhydrazyl) radical scavenging activity;

MC: Metal chelating activity;

FRAP: Ferric reducing power assay

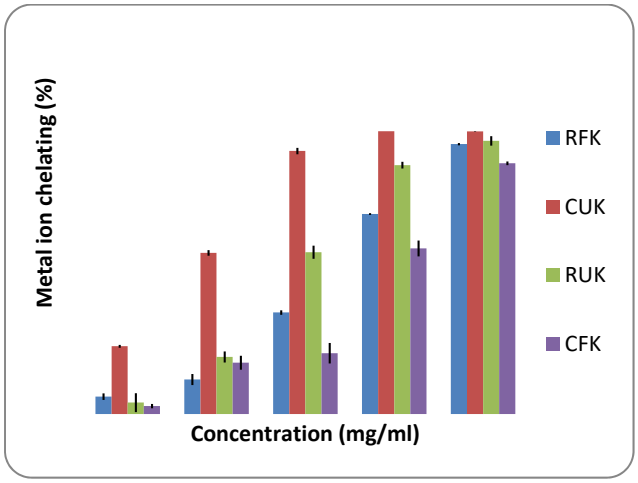

Figure 3: Metal chelating effects of kariya seed defatted flour at different concentrations

Considering the effect of the processing methods on the reducing power of this present study, the extracts of sample CFK (0.56ug/g) registered the maximum level of reducing power followed by sample RFK (0.28ug/g). The result obtained in this study reveals that sample CFK had better ability to transfer electrons. This result is similar to that obtained by Asirvatham *et al*., 2011 for the antioxidant activity of raw and differentially processed underutilized legumes *Canavalia ensiformis* seeds, where it was seen that extracts of cooked samples registered the maximum level of reducing power. Juntachote (2005) revealed that samples with higher reducing power have better abilities to donate electron to free radicals to form stable substances, thereby interrupting the free radical chain reactions. Hence, the consumption of cooked fermented and fermented kariya seeds may be of beneficial effect.

3.9. Total phenol and flavonoid content

Antioxidant compounds generally contain phenolic groups. Phenols are very important plant constituents which exhibit anti-oxidative properties because of their scavenging ability due to their hydroxyl groups (Diplock, 1997). It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Cook and Samman, 1996; Kessler *et al*., 2003). The total phenolic and flavonoid contents of all the samples are shown in Table 2. The result obtained in this study reveals that samples RFK and CFK extracts had significant ($P < 0.05$) higher total phenol and flavonoids contents (1.21, 0.90 and 0.14, 0.08) than did samples CUK and RUK (0.60, 0.47 and 0.00, 0.03). The values of the TPC obtained in this study were higher than those of African yam bean hydrolysates (12.86 to 49.44 and 4.75 to 35.26 GAE/g) as reported by Fasasi *et al*., 2012. According to Shaidi and Naezk (2004), natural phenolics exert beneficial effects mainly through their antioxidant activity. These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial radical, such as hydroxyl radicals, chelating metal ion catalyst, decomposing primary oxidation products to non-radical species and breaking chains to prevent continued hydrogen abstraction from substances (Enujiugha, 2010) .Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Nabavi *et al*., 2009a). The high amount of phenols and flavonoids in the extracts may explain their high antioxidant activities and this may help in the prevention of various diseases associated with oxidative stress.

4. CONCLUSION

This research has shown that fermented and unfermented Kariya seed flour could serve as a good source of protein ingredient in food systems as seen with an increase in the water absorption capacity. Therefore fermented kariya flour could be incorporated into food formulations especially those involving dough handling. The decrease in bulk density as a result of fermentation would also make the fermented products from kariya a useful ingredient in infant formulations which nutritionally could promote easy digestibility of food products where it is incorporated especially for children with immature digestive system. Fermented kariya crude extract showed higher antioxidant potency than raw crude extract. These results clearly indicate that fermentation may be useful in improving the nutritional quality of kariya with respect to protein as a functional ingredient in many food products.

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