EFFECT OF Moringa oleifera LEAF POWDER INCLUSION ON THE PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF AKAMU

Asogwa, I.S. and Ani, J.C.
Department of Food Science and Technology, University of Nigeria, Nsukka, Nigeria
email: ifeyinwas.asogwa@unn.edu.ng

ABSTRACT
Moringa leaf powder (MLP) was added akamu at 10%, 20%, 30% and 40% levels of incorporation and labelled MA₁, MA₂, MA₃ and MA₄ respectively. Another batch of MLP was blended with an equal amount of soybean flour (SBF) and similarly incorporated into akamu at the same levels and labelled MSA₁, MSA₂, MSA₃ and MSA₄. Akamu supplemented with 40% soybean flour (SBA) and unsupplemented akamu (AKM) served as controls. The total phenolic content of akamu increased from 0.35 to 3.06 g/100g, flavonoids increased from 0.06 to 0.27 g/100g for AKM and MA₄ respectively. The total phenolic content for SBF incorporated akamu increased (p < 0.05) from 0.35 g/100g to 1.71 g/100g, flavonoids increased (p < 0.05) from 0.06 g/100g to 0.25 g/100g for AKM and MSA₄ respectively. The percentage DPPH scavenging activity of akamu was enhanced significantly (p < 0.05) by the addition of MLP and SBF. The DPPH activity of MA₄ (88.91%) was significantly higher than that of the AKM (33.40%). Similarly, the DPPH activity of MSA₄ (86.34%) was higher than that of AKM. There were significant (p < 0.05) increases in reducing power with levels of addition of both MLP and SBF. The reducing power was elevated from 2.30 mg/g for AKM to 28.8 mg/g for MA₄ and to 39.9 mg/g for MSA₄. The inclusion of moringa leaf powder (with or without soybean flour) was able to significantly increase the antioxidant activity of akamu, thus enhancing its functional status.

Key words: Moringa leaf powder, soybean flour, phytochemicals, antioxidant activity

INTRODUCTION
The National Academy of Sciences’ Food and Nutrition Board defined functional foods as “any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains” (IOM/NAS, 1994). A food can also be said to be functional if it contains a component (whether or not a nutrient) that benefits one or a limited number of functions in the body in a targeted way that is relevant to either the state of well-being and health or the reduction of the risk of a disease (Bellisle et al., 1998) or if it has physiologic or psychologic effect beyond the traditional nutritional effect (Clydesdale, 1997). The major basis for the prevalent interest in functional foods comes from the rather consistent findings that increased fruit and vegetable consumption is accompanied by a reduction in the risk of heart disease and cancer (Craig, 1997; de Lorgeril et al., 1998; Pereira et al., 2004; Boeing et al., 2012). This is because plants are rich in phytochemicals which are bioactive compounds that have been linked to reduction in the risk of major degenerative diseases. This health promoting ability is majorly due to their function as antioxidants (Prakash et al., 2007a,b; Sreelatha and Padma, 2009; Lobo et al., 2010). These antioxidants are able to reduce oxidative stress caused by the accumulation of free radical and reactive oxygen species in the body. Oxidative stress leads to the progressive accumulation of oxidative damage in tissues resulting in a sequence of events that may lead to the dysfunction of specific cells, tissues or organs. Oxidative stress has been implicated as a mediator of degenerative and chronic deteriorative, inflammatory and autoimmune diseases (Heliovaara et al., 1994; Nice, 1997). Diabetes, vascular disease and hypertension (Gey, 1986; Harris, 1992), cancer and hyperplastic diseases (Ames et al., 1993; Ferguson, 1994), arthritis, malaria (Nice, 1997), brain dysfunction as well as the ageing process (Ames et al., 1993) have all been linked to oxidative stress. There are many ways to make a food functional including increasing the concentration of a component naturally present in food to a point at which it will induce predicted effects (e.g., fortification with a micronutrient) to reach a daily intake higher than the recommended daily intake but compatible with...
the dietary guidelines for reducing risk of disease (Block, 1993) or increasing the concentration of a nonnutritive component to a level known to produce a beneficial effect or by adding a component that is not normally present in most foods and is not necessarily a macronutrient or a micronutrient but for which beneficial effects have been shown eg, non-vitamin antioxidant (Roberfroid, 2000). Akamu is among the staples of the populace in Nigeria and some other African countries. This fermented cereal product is also known as koko in Northern Nigeria (Bristone et al., 2016), ogi, uji, and akosa in Southwest Nigeria, Kenya, and Ghana, respectively (Adegbehingbe, 2013). Akamu is a Nigerian traditional lactic acid fermented cereal-based meal, made basically from maize (Zea mays) and other cereals; sorghum or millet (Akingbala et al., 1981; Teniola and Odunfa, 2001; Inyang and Idoko, 2006; Ogodo et al., 2015). Akamummeal is served in Nigeria as a complementary food for infants and a breakfast food for children and adults. Unfortunately, akamu has been reported to be deficient in essential nutrients for the growth of infants (Ikujenlola and Adurotuye, 2014). This is because similar to all cereals, maize or sorghum used traditionally to produce akamu has two significant flaws. Firstly, it is low in protein (9-10%) and secondly, it does not provide the essential amino acids (lysine and tryptophan) in sufficient quantities to meet the nutritional needs of infants (Vasal, 2006; Omage et al., 2009). Since the majority of people living in the rural areas cannot afford milk based infant formula for supplementation of akamu, the search for cheaper alternatives became very necessary. It is in this light that Akinrele and Edwards (1971) enriched ogi with soya bean to form a product known as soy-ogi, which has wide acceptability in Nigeria. It is a good source of protein because of the added soya bean, and consequently is used in complementary feeding. A lot of studies have further been done on supplementing akamu with other legumes (Ikujenlola and Adurotuye, 2014; Ikujenlola et al., 2013; Bristone et al., 2016). Little information, however, is available on increasing the potential of akamu as a functional food by the addition of food ingredients especially the easily available and affordable ones. Odunlade et al. (2016) reported that the antioxidant activity and the total phenolic component of akamu was improved by the addition of cocoa powder. There is need to investigate the effect of addition of more affordable plant sources on the phytochemical and antioxidant activity of akamu thereby enhancing the health promoting potential of this staple food. *Moringa oleifera* has been reported to be a good source of natural antioxidants due to the presence of phytochemicals like ascorbic acid, flavonoids, phenolics and carotenoids (Anwar et al., 2005; Makkar and Becker, 1996). Monica premiet al., 2010 had reported that *Moringa oleifera* leaves are very rich in phytochemicals that have potent antiinflammatory and hypotensive activities. Moringa leaf is known to be beneficial for people with cardiovascular disorders. The leaves have been reported to have hypocholesterolaemic property (Talahalian and Kar, 2000). Its leaf juice is also known to have a stabilizing effect on blood pressure (Gilani et al., 1994; Fuglie, 1999; Olugbemi et al., 2010). It is known to be helpful for people with diabetes mellitus and is used to treat gastric ulcers (Pal et al., 1995). The leaves have been reported to possess gastric cytoprotective effect (Cas et al., 2000). The antibiotic *Eilert et al., 1981* and antimicrobial properties of the leaves have also been reported (Palaniswamy, 2005). It is against this background that this present study was designed to investigate the effect of moringa leaf powder inclusion in *akamu* (with or without soy bean flour) on the phytochemical and antioxidant status of akamu.

**MATERIALS AND METHODS**

**Moringa leaf powder preparation**

Fresh moringa leaves were collected from a farm in Gboko, Benue State, Nigeria. The leaves were stripped off, washed thoroughly, allowed to drain and then spread thinly on aluminum trays in a well ventilated room (27°C ± 1) to dry for five days. The shade dried leaves were ground (hammer mill, Thomas Willey mills, model Ed-5, Germany), sieved with a screen of 2 mm pore size, placed in amber coloured glass containers and stored in the refrigerator (−4°C) for blending.

**Soybean flour preparation**

Soybean seeds were sorted and thoroughly washed with clean tap water and then boiled in excess water for 40 mins. The beans were drained and soaked in excess water for 12 h and the water was changed every 3h. Thereafter, the seed coats were removed by rubbing with hands. The cotyledons were separated from the seed coats and the seeds spread on aluminum trays and dried under the sun (32°C ±1) for 2 days. The dried seeds were milled in a commercial attrition mill into flour and sieved with a muslin cloth of 1mm pore size (Enwere, 1998).
Akamu preparation

Akamu was prepared from the white variety of dry maize purchased from Nsukka main market, Nsukka, Enugu State, Nigeria. The flow chart for akamu preparation is shown on Figure 1.

Blending formula

Soybean flour (SBF) and MLP were blended in the ratio of 1:1. The MLP and soybean flour/MLP (SBF/MLP) blends were added to the dried akamu at different levels of incorporation as shown on Table 1.

Extraction of total phenolic content

The method described by Eberhardt et al. (2000) was used. Extraction was done on 25 g of each sample using 50 ml of 80% chilled ethanol after centrifugation for 10 mins after centrifugation (Gallenkamp centrifuge, England) at 2500 rpm. The supernatant was decanted and extraction repeated one more time. Supernatants were pooled and the volume of extract reduced to 10 ml in a flash evaporator at 45°C. The volume of the extract was made up to 25 ml with distilled water and was stored at -4°C until use.

Determination of total polyphenol content

The total phenolic content was determined using the Folin-Ciocalteu assay (Ragazzi and Veronese, 1973). To 1 ml of extract, 10 ml of deionized water and 2 ml of Folin-Ciocalteu phenol reagent were added. The mixture was allowed to stand for 5 mins and then it was allowed to cool for 10 mins in a water bath maintained at room temperature. Absorbance was read with a spectrophotometer (Spectro21D, Pec Medicals, USA) at 420 nm.

Extraction of total flavonoids

Extraction was done by the method described by Siddhuraju and Becker, (2003). One gram of each sample was extracted in an apparatus containing a round-bottom flask and reflux condenser with 100 ml of 80% methanol for 3h and the extract filtered. The volume of the extract was made up to 100 ml with 80% methanol.

Determination of flavonoid content

Total flavonoid content was determined by a colorimetric assay developed by Zhishen et al. (1999). A 1 ml aliquot of appropriately standard solutions of catechin was added to a 10 ml volumetric flask containing 4 ml distilled water. At zero time 0.3 ml NaNO₃ was added to the flask. After 5 mins, 0.3 ml 10% AlCl₃ was added. At 6 mins, 2 ml of 1M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 ml of double distilled H₂O and thoroughly mixed. Absorbance of the developed pink colour of the mixture was determined at 510 nm against a blank. Total flavonoids was expressed as catech in equivalent.

Determination of saponin content

This was done using the method of Uematsu et al. (2000). One gram of sample was extracted with 20 ml of ethanol and shaken for 10 mins, the mixture was centrifuged (Gallenkamp centrifuge England) at 3000 rpm for 10 mins. Thereafter, 2 ml of the supernatant was placed in a test tube and evaporated over a boiling water bath. After cooling, 2 ml of ethyl acetate, 1 ml of 0.5 ml anisaldehyde and 99.5 ml ethyl acetate were added. Thereafter, 1 ml of a mixture of 50 ml concentrated H₂SO₄ and 50 ml ethyl acetate were added. After stirring, the test tube was placed in a water bath at 60°C for 20 mins and then it was allowed to cool for 10 mins in a water bath maintained at room temperature. Absorbance was read with a spectrophotometer (Spectro21D, Pec Medicals, USA) at 430 nm.

Determination of steroid content

Steroid content was determined with the method described by Okeke and Elekwa (2003). A 0.5 g weight of each sample was dispersed in 100 ml freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered and the filtrate eluted with normal ammonium hydroxide solution (pH 9). The eluate (2 ml) was mixed with 2 ml of chloroform and 3 ml of ice cold acetic anhydride was added. Thereafter, 2 drops of concentrated H₂SO₄ was cautiously added. Standard steroid solution was prepared and treated as described above. The absorbance of standards and prepared samples was measured in a spectrophotometer (Spectro21D, Pec Medicals, USA) at 420 nm.

Evaluation of reducing power

The reducing power of the 80% methanolic sample extracts was determined according to the method of Yen and Chen (1995). The extract was mixed with an equal volume of 0.2M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 mins after which the equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged (Gallenkamp centrifuge, England) at 3000 rpm for 10 mins. The upper layer of the solution was mixed with distilled water and 0.1% FeCl₃ in a ratio of 1:1:2 and the
absorbance of the upper layer was measured at 700 nm using a spectrophotometer (Spectro 21D, Pec Medicals, USA). Increased absorbance of the reaction mixture indicated increase in reducing power. Total reducing power was expressed as absorbance units per total phenolics per gram of sample.

**Evaluation of scavenging activity against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical**

This was carried out according to the DPPH spectrophotometric method of Mensor et al. (2001). One ml of a 0.3 mM DPPH methanol solution was added to 2.5ml solution of the 80% methanolic sample extract or standard and allowed to react at room temperature for 30mins. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA) using the formula:

\[
AA\% = 100- \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

One ml of 0.3mM DPPH plus 2.5ml 80 % methanol was used as control.

**Data analysis**

Quantitative data were expressed as means and standard deviation (SD) of at least three measurements. Each experimental set was compared with one way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS) version 15 (SPSS Inc., Chicago, IL, USA). Duncan’s new multiple range test was used to determine the differences between means. The values p<0.05 were regarded as significant.

**RESULTS AND DISCUSSION**

The data on the effects of MLP addition on the phytochemical content of *akamu* are presented on Table 2, while the data on the effects of MLP/SBF addition on the phytochemical content of *akamu* is presented on Table 3. The different levels of addition had significant (p<0.05) effects on all the phytochemical components evaluated. Addition of either MLP alone or a blend of MLP/SBF led to significant (p<0.05) increases in the phytochemical levels. For instance, the phenolic content varied from 1.56 g/100g for MA1 to 3.06 g/100g for MA4 while the steroid content progressed from 0.59 to 1.94 mg/100g for MA1 and MA4, respectively. Similarly, the phenolic and steroid content of the MSA samples ranged from 0.91 to 1.71 g/100g and 1.95 to 2.27mg/100g for MSA1 and MSA4, respectively. The MA samples had higher levels of total phenols, flavonoids and saponin because these components were higher in MLP than soy bean flour. On the other hand, MSA samples were higher in steroid because soy bean flour has higher steroid than MLP. Odunlade et al. (2016) reported an increase in the total phenolic content of *akamu* with increasing level of cocoa powder addition. The elevation in the total phenolic content of *akamu* due to the addition of either MLP or MLP/SBF blends implies that its antioxidant activity will be elevated. Antioxidants are known to be beneficial in the reduction of oxidative stress that is associated with the etiology of many cardiovascular and degenerative diseases.

**Fig 1:** Preparation of *akamu*

**Source:** Alabi and Anuonye (2007)

**Figure. 2:** Effect of MLP addition on % DPPH activity of *akamu*
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Table 1: Blending ratios for MLP and SBF/MLP incorporation in dried akamu

| Sample code | Akamu % | MLP % | SBF % | P % |
|-------------|---------|-------|-------|-----|
| MA          | 90      | 10    | -     |     |
| MA          | 80      | 20    | -     |     |
| MA          | 70      | 30    | -     |     |
| MA          | 60      | 40    | -     |     |
| MA          | 50      | 50    | -     |     |
| MA          | 40      | 60    | -     |     |
| MA          | 30      | 70    | -     |     |
| MA          | 20      | 80    | -     |     |
| MA          | 10      | 90    | -     |     |
| MA          | 0       | 100   | -     |     |

Table 2: Effect of addition of MLP on the phytochemical content of akamu

| Sample | Total phenol g/100g | Flavonoid mg/100g | Steroid mg/100g | Saponin mg/100g |
|--------|---------------------|-------------------|----------------|-----------------|
| MA     | 1.56±0.13           | 0.17±0.007        | 0.59±0.043     | 37.25±0.02      |
| MA     | 2.26±0.08           | 0.18±0.002        | 1.43±0.111     | 41.75±0.00      |
| MA     | 2.86±0.15           | 0.21±0.003        | 1.51±0.090     | 42.64±0.02      |
| MA     | 3.06±0.02           | 0.27±0.004        | 1.94±0.026     | 42.73±0.00      |
| SBA    | 0.49±0.08           | 0.07±0.004        | 2.82±0.04      | 41.93±0.03      |
| AKM    | 0.35±0.04           | 0.06±0.002        | ND             | ND              |

Effect of addition of MLP and MLP/SBF blends on percentage DPPH scavenging activity of akamu

Figures 2 and 3 show the effect of MLP addition and MLP/SBF blend on the percentage DPPH scavenging activity of akamu. There were significant (p<0.05) increases in the %DPPH scavenging activity of the samples as a result of MLP and MLP/SBF blend addition. The percentage DPPH activity of the MA samples ranged from 86.08% for MAa to 88.91% for MAb. These values are significantly lower (p<0.05) than 92.52% for the butylated hydroxyl toluene (BHT), a commercial antioxidant but much higher than 66.91% for ascorbic acid. The %DPPH scavenging values for the MSA samples ranged from 63.66% for MSAa to 86.34% for MSAb. The % DPPH for akamu samples containing MLP alone were higher (p<0.05) than those of akamu samples containing blends of MLP and soybean flour. This observation implied that MLP is rich in antioxidants especially phenolic compounds (Anwar et al., 2005). Many phenolics such as flavonoids have antioxidant capacities that are much stronger than those of vitamins C and E. They also possess free radical scavenging ability (Amic et al., 2003). The antioxidant activities of plant phytochemicals occur by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing/chelating the transition metal composition of foods (Melo, 2006; Obbo et al., 2007). Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action (Dastmalchi et al., 2007).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compounds extracts with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears (Brand-Williamset al., 1995). Shyamala et al. (2005) reported free radical scavenging activities of greater than 70% for three green leafy vegetables namely Spinacia oleracea, Coriandrum sativum and Alternanthera sessilis at 100ppm levels. The higher % DPPH activity of both the MA samples and most of the MSA samples than MLP alone suggests a synergistic action between components of akamu and those of MLP and should be investigated further.

Effect of addition of MLP and MLP/SBF blends on ferric reducing antioxidant property (FRAP) of akamu

Effect of addition of MLP and MLP/SBF blends on ferric reducing antioxidant property (FRAP) of akamuis shown in figures 4 and 5. The reducing power scores ranged from 11.7 mg/g for MAa to 30.9 mg/g for MAb and 21.30 mg/g to 39.90 mg/g for the MSA samples. These values are higher than the reducing power of BHA (11.6 mg/g) but lower than that of ascorbic acid (41.1 mg/g). Reducing power is part of the antioxidation defense mechanism. The two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). The higher reducing power of the MSA samples over the MLP samples could also suggest a synergistic action between the MLP components and soy bean flour components. Further research is therefore needed to investigate this observation.
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**Table 3:** Effect of addition of MLP/SBF flour blends on the phytochemical content of *Akamu*

| Sample          | Total phenol (mg/100g) | Flavonoid (mg/100g) | Steroidal (mg/100g) | Saponin (mg/100g) |
|-----------------|------------------------|---------------------|---------------------|-------------------|
| MSA1            | 0.91±0.006             | 0.098±0.017         | 1.95±0.004          | 32.5±0.03         |
| MSA2            | 1.28±0.08              | 0.16±0.003          | 1.96±0.013          | 37.0±0.02         |
| MSA3            | 1.51±0.02              | 0.18±0.017          | 2.12±0.006          | 37.84±0.05        |
| MSA4            | 1.71±0.12              | 0.25±0.004          | 2.27±0.029          | 42.20±0.02        |
| SBA             | 0.49±0.08              | 0.075±0.004         | 2.82±0.04           | ND                |
| AKM             | 0.35±0.04              | 0.06±0.002          | ND                  | ND                |

MSA – *Akamu* supplemented with 10% MLP/SBF blend, MSA1 - *akamu* supplemented with 20% MLP/SBF blend, MSA2 – *akamu* supplemented with 30% MLP/SBF blend, MSA3 – *akamu* supplemented with 40% MLP/SBF blend, SBA - *akamu* supplemented with 40% soy bean flour, AKM- unsupplemented *akamu*. ND- not detected. Values on the same column bearing different superscripts are significantly different (p<0.05).

**CONCLUSION**

This study showed that addition of moringa leaf powder either alone or as a blend with soybean flour is able to appreciably increase the phytochemical content of *akamu*. This led to increase in its antioxidant properties. It can be concluded that the functionality of *akamu* would be improved by the addition of moringa leaf powder alone or in combination with soybean flour.

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