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

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ABSTRACT
Moringa leaf powder (MLP) was added akamu at 10%, 20%, 30% and 40% levels of incorporation and labelled MA_1, MA_2, MA_3 and MA_4 respectively. Another batch of MLP was blended with an equal amount of soybean powder (SBF) and similarly incorporated into akamu at the same levels and labelled MSA_1, MSA_2, MSA_3 and MSA_4. 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 MA_4, 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 MSA_4 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 MA_4 (88.91%) was significantly higher than that of the AKM (33.40%). Similarly, the DPPH activity of MSA_4 (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 MA_4 and to 39.9 mg/g for MSA_4. 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 e*t 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 (Heliövaara*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

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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 Africancountries. 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). *Akamu*meal 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 Adurotoye, 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 Adurotoye, 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 compoment 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 premi*et al*, 2010 had reported that *Moringaoleifera* leaves are very rich in phytochemicals that have potent antitumour and hypotensive activities. Moringa leaf is known to be beneficial for people with cardiovascular disorders. The leaves have been reported to have hypocholesterolaemic property (Talhaliani and Kar, 2000). Its leaf juice is also known to have a stabilizing effect on blood pressure (Gilani *et al.,* 1994).*Moringa oleifera* was claimed to boost immune systems (Jayavardhanan *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 (Casa *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 ($27^oC \pm 1$) 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^oC) 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 ($32^oC \pm 1$) 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 was 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 minsafter 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^{o}C. The volume of the extract was made up to 25 ml with distilled water and was stored at -4^{o}C until use.

Determination of total polyphenol content
The total phenolic content was determined using the Folin-Ciocalteu assay (Ragazzi and Veronese, 1973). To 1ml 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 2 ml of sodium carbonate was added. The absorbance (Spectro21D, Pec Medicals, USA) of blue complex was read at 750 nm. The total phenolic content was calculated from the calibration curve prepared from the absorbance of tannic acid standard solutions.

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 100ml with 80% methanol.

Determination of flavonoid content
Total flavonoid content was determined by a colorimetric assay developed by Zhishen *et al.* (1999). A 1ml aliquot of appropriately standard solutions of catechin was added to a 10ml volumetric flask containing 4 ml distilled water. At zero time 0.3 ml $NaNO_3$was added to the flask. After 5 mins, 0.3 ml 10 % $AlCl_3$ 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 H_2O 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 H_2SO_4 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 H_2SO_4 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 an 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% $FeCl_3$ 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-picryhydrazyl (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- [(Absorbance of sample/Absorbance of control) x 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 MA_1 to 3.06 g/100g for MA_4 while the steroidcontent progressed from 0.59 to 1.94 mg/100g for MA_1 and MA_4, respectively. Similarly, the phenolic and steroid content of the MSA samples ranged from 0.91to 1.71 g/100g and 1.95 to 2.27mg/100g for MSA_1 and MSA_4, 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.

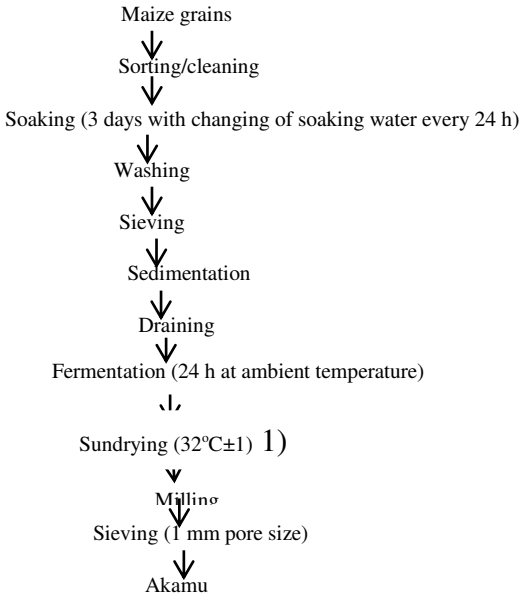

Fig 1: Preparation of *akamu*
Source: Alabi and Anuonye (2007)

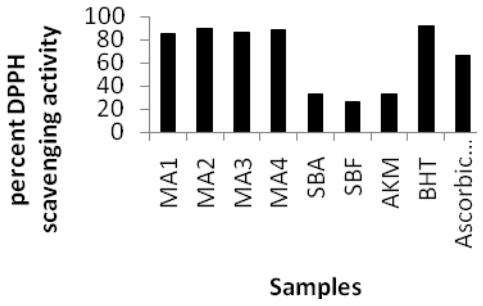

Figure. 2: Effect of MLP addition on % DPPH activity of *akamu*

Table 1:Blending ratios for MLP and SBF/MLP incorporationin dried *akamu*

Sample	Sample code	Akamu %	MLP	SBF /MLP %
Akamu with 10% MLP	MA_1	90	10	-
Akamu with 20% MLP	MA_2	80	20	-
Akamu with 30% MLP	MA_3	70	30	-
Akamu with 40% MLP	MA_4	60	40	-
Akamu with 10% SBF/MLP	MSA_1	90	-	10
Akamu with 20% SBF/MLP	MSA_2	80	-	20
Akamu with 30% SBF/MLP	MSA_3	70	-	30
Akamu with 40% SBF/MLP	MSA_4	60	-	40
Akamu with 40% SBF	SBA	60	-	40
Akamu alone	AKM	100	-	-

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

Sample	Total phenol g/100g	Flavonoid g/100g	Steroid mg/100g	Saponin mg/100g
MA_1	1.56^{c}±0.13	0.17^{d}±.007	0.59^{d}±0.043	37.25^{g}±0.02
MA_2	2.26^{b}±0.08	0.18^{c} ± 0.002	1.43^{c}±0.011	41.75^{e}±0.00
MA_3	2.86^{a}±0.15	0.21^{b}±0.003	1.51^{c}±0.090	42.64^{c}±0.02
MA_4	3.06^{a}±0.02	0.27^{a} ±0.004	1.94^{b}±0.026	42.73^{b}±0.00
SBA	0.49^{f}±0.08	0.075^{f} ±0.004	2.82^{a}±.04	41.93^{d}±0.03
AKM	0.35^{d}±0.04	0.06^{h} ±0.002	ND	ND

MA_1–*Akamu* supplemented with 10% MLP, MA_2– *akamu* supplemented with 20% MLP, MA_3 – *akamu* supplemented with 30% MLP, MA_4 – *akamu* supplemented with 40% MLP, 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 ($p<0.05$)

Effect of addition of MLP and MLP/SBF blend 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 MA_1 to 88.91% for MA_4. 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 MSA_1 to 86.34% for MSA_4. 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; Oboh *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).

DPPHis a stable free radical and accepts an electron orhydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compoundsor extracts with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a freeradical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radicalat 517 nm, but upon reduction by an antioxidant, the absorption disappear (Brand-Williams*et 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 *akamu*is shown in figures 4 and 5.The reducing power scores ranged from 11.7 mg/g for MA_1 to 30.9mg/g for MA_3 and 21.30 mg/g to 39.90mg/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.

Table 3: Effect of addition of MLP/SBF flour blends on the phytochemical content of *akamu*

Sample	Total phenol	Flavonoid	Steroidm	Saponin
	g/100g	g/100g	g/100g	mg/100g
MSA_1	0.91^{c}±0.006	0.098^{d}±0.017	1.95^{d}±0.004	29.23^{d}±0.03
MSA_2	1.28^{b}±0.08	0.16^{c}±0.003	1.96^{d}±0.013	37.80^{c}±0.02
MSA_3	1.51^{ab}±0.02	0.18^{b}±0.017	2.12^{c}±0.006	37.84^{c}±0.05
MSA_4	1.71^{a}±0.12	0.25^{a}±0.004	2.27^{b}±0.029	42.20^{a}±0.02
SBA	0.49^{d}±0.08	0.075^{e} ±0.004	2.82^{a}±.04	41.93^{b}±0.03
AKM	0.35^{e}±0.04	0.06^{f} ±0.002	ND	ND

MSA_1 – *Akamu* supplemented with 10% MLP/SBF blend, MSA_2 – *akamu* supplemented with 20% MLP/SBF blend, MSA_3 – *akamu* supplemented with 30% MLP/SBF blend, MSA_4 – *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 ($p<0.05$)

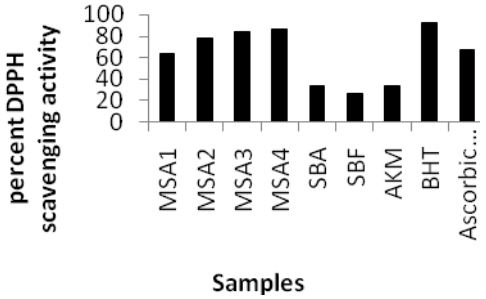

Figure. 3: Effect of MLP/SBF blend addition on % DPPH activity of *kamu*

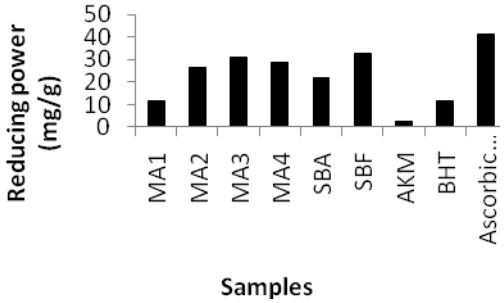

Figure. 4: Effect of MLP addition on the ferric reducing antioxidant property (FRAP) of *akamu*

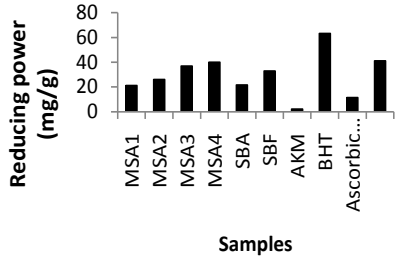

Fig. 5: Effect of MLP/SBF blend addition on the ferric reducing antioxidant property (FRAP) of *akamu*

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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