

Full Length Research Paper

Enrichment of traditional maize snack (Kokoro) with moringa (*Moringa oliefera*) leaf and soybean

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Kokoro, a local maize snack was made from white maize (W) flour and supplemented with *Moringa oliefera* leaf (M) and defatted soybean (S). WMS₀ (100:0:0%), WMS₁ (90:10:0%), WMS₂ (90:0:10%), WMS₃ (90:5:5%), WMS₄ (90:7.5:2.5%), and WMS₅ (90:2.5:7.5%) were carried out in triplicates. Kokoro was produced by deep frying in hot refined vegetable oil. The proximate composition, vitamin content and phytochemicals composition were determined. Kokoro formulated with 90% maize flour and 10% defatted soybean (WMS₂) had the highest moisture, crude protein, fat, oxalate, phytic acid and alkaloid, while Kokoro formulated with 90% maize flour and 10% moringa (WMS₁) had the highest crude fibre, vitamin A, B₃ (niacin), C and flavonoid. On the other hand, Kokoro formulated with only 100% maize flour (WMS₀) had the least phytochemical composition and vitamins A, B₃, C contents. Although, the addition of soybean had the highest positive effect on the protein and crude fibre contents of Kokoro, it was the addition of moringa that had the highest positive effect on the vitamins contents. On the other hand, moringa also raised the phytochemical contents significantly ($p \leq 0.05$). Overall, sample WMS₄ (90% Maize + 7.5% Moringa + 2.5% Soybean) had substantial proximate and minerals composition in addition to having the least phytochemical could be considered the best formulation for Kokoro formulation.

Key words: Moringa, phytochemicals, proximate composition, soybean, Kokoro.

INTRODUCTION

The traditional maize (*Zea mays* L. Poaceae) snack (Kokoro) is a popular local snack in Southwestern Nigeria and is made solely from maize flour that contains primarily carbohydrates (Awolu et al., 2016a). Snack containing 100% maize do not meet nutritional requirements of the body, hence the need to fortify with

crops rich in protein, fibre, minerals and antioxidants (Awolu et al., 2016a, 2015; Omuetti and Morton, 1996). Specifically, toasted had been produced by optimum blends using response surface methodology (Awolu et al., 2016b).

Maize, the primary material for making Kokoro snack is

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a cereal and is deficient in the essential amino acids such as lysine and tryptophan (Omuetti et al., 1992) that are essential for human nutrition (Lasztity, 1984). Research effort has been concentrated on supplementing cereal food with legumes and has successfully enhanced the nutritional value and/or functionality of staple foods (Awolu et al., 2016a).

Moringa oleifera is a highly valuable plant, distributed in many tropical and subtropical countries. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source for protein, vitamins, β -carotene, amino acids and various phenolics. Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals. It is also very important plant for its medicinal value. Various parts of the plant such as leaves, roots, seeds, bark, fruit, flowers and immature pods, etc., as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, antibacterial and antifungal (Bukar et al., 2010). *M. oleifera* leaves are highly nutritious. In 100 g dry matter, they contain 29 ± 6 g of protein, 28 ± 6 mg of iron, $1,924 \pm 288$ mg of calcium, $15,620 \pm 6,475$ IU of vitamin A and 773 ± 91 mg of vitamin C. This is at least twice the protein in milk and half the protein in egg, and has more iron than in beef, more calcium than in milk, equal vitamin A to carrot and more vitamin C than in orange (Wangcharoen and Gomolmanee, 2013). In addition, the leaves of this plant are reported to have various biological activities such as diuretic, immune boosting and hypotensive, anti-inflammatory, antiulcer, antihepatotoxic, antitumour, thyroid hormone status regulating, hypocholesterolaemic, radioprotective, hypolipidaemic, antiatherosclerotic, antidiabetic, and antioxidant (Jaiswal et al., 2009; Chumark et al., 2008; Singh et al., 2009; Sreelatha and Padma, 2008; Verma et al., 2009).

This study was to evaluate the addition of soybean and moringa into maize for the production of Kokoro with high nutritional and phytochemical properties.

MATERIALS AND METHODS

Sample collection and preparation

Maize (*Zea mays* L., Poaceae) was sourced from the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. It was shelled, winnowed and sorted to remove damaged grains before milling. Soybean (*Glycine max* (L.) Merrill) was sourced from Ondo State Agricultural Development Project (ADP), Akure. The seeds were cracked with hammer mill and seed coat winnowed. It was toasted, cooled and milled into flour. The flour was defatted with n-hexane in a Soxhlet apparatus. *Moringa* leaf was plucked from the branch of the tree raised in Akure, Nigeria. The leaves were cleaned, air dried under shade, dry milled and kept in airtight container. Considerable amount of chlorophyll in the leaves were removed by soaking in ethanol overnight, followed by

draining and air-drying.

Composite flour formulation

White maize (W), moringa (M) and soybean (S) were mixed to make 180 g at different ratios and replicated three times in the following percentage: WMS₀ (100:0:0%), WMS₁ (90:10:0%), WMS₂ (90:0:10%), WMS₃ (90:5:5%), WMS₄ (90:7.5:2.5%), and WMS₅ (90:2.5:7.5%). Each of the mixture was replicated three times.

Formulation of Kokoro

Salt was added to 180 g composite flour to taste and was gently stirred into 0.5 L boiling water in a stainless steel pot. The mixture was cooked with continuous stirring until stiff dough was formed. The dough was cooled to a temperature (40°C) at which it could be kneaded by hand for 5 min. The kneaded dough was cut into pieces, rolled into cylindrical shapes and deep fried at 150°C in 1 L of hot refined vegetable oil for 3 min. The fried Kokoro was then cooled and packed in sealed polyethylene bags. The frying was carried out in triplicates.

Proximate analysis of Kokoro

Moisture and protein content were determined using the procedure described by AOAC (1990). The ash content was determined using the procedure described by Pearson (1976).

The fat content determination was carried out by soxhlet extraction method (AOAC, 2005). Oil in 5 g sample was extracted using hexane in Soxhlet extraction equipment for 2.5 h under reflux. The crude fibre content was determined using the procedure described by Kirk and Sawyer (1991). Total carbohydrate was estimated using the formula (Ouzouni et al., 2009):

Total carbohydrates (% f) = [100 - moisture (%) - protein content (%) - crude fat (%) - ash (%)].

Determination of vitamin A

Method of AOAC (2005) was used. Exactly 1 ml of the hydrophilic extracts from the sample was measured to the test-tube I (centrifugal) with a tight stopper and 1 ml of the KOH solution was added, the tube was plugged and shake vigorously for 1 min. The tube was heated in a water bath (60°C, 20 min), and was then cooled down in cold water. About 1 ml of xylene was added, the tube was plugged and shake vigorously again for 1 min. The tube was centrifuged (1500xg, 10 min), the whole of the separated extract (upper layer) was collected and transferred into the test tube II made of "soft" (sodium) glass. The absorbance A₁ of the obtained extract was measured at 335 nm against xylene. The extract in the test tube II was irradiated to the UV light for 30 min, then the absorbance A₂ was measured. The concentration (Cx) of vitamin A (μ M) in the analyzed liquid was calculated using the equation:

$$Cx = [A_1] - [A_2] \cdot 22.23$$

where 22.23 is the multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette, 1 cm thickness.

Determination of vitamin B₃ (Niacin)

Five grams of the sample was treated with 50 ml 1N H₂SO₄ and

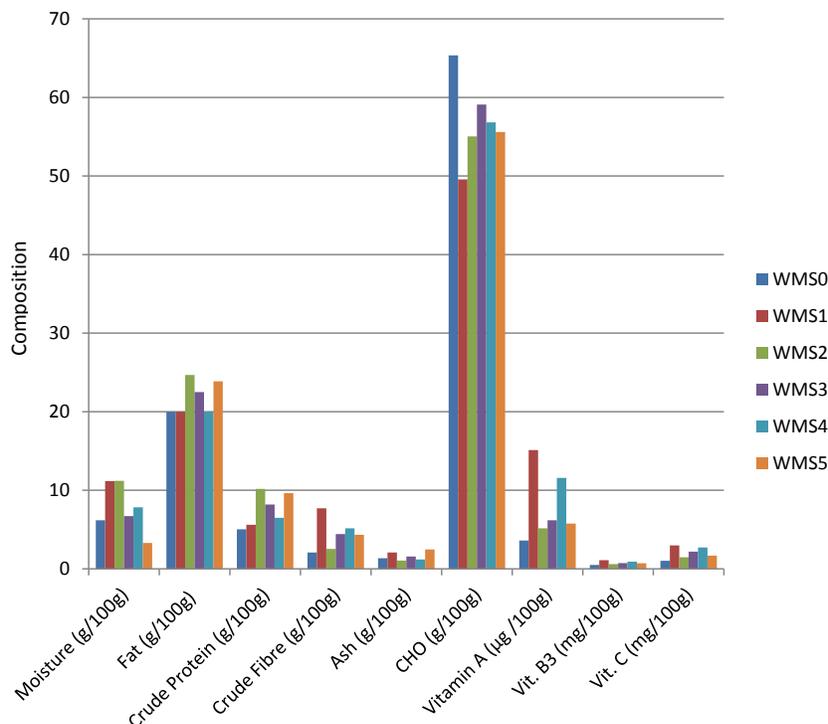


Figure 1. Proximate composition of formulated Kokoro. WMS₀ (100% Maize flour), WMS₁ (90% Maize + 10% *Moringa* + 0% Soybean), WMS₂ (90% Maize + 0% *Moringa* + 10% Soybean), WMS₃ (90% Maize + 5% *Moringa* + 5% Soybean), WMS₄ (90% Maize + 7.5% *Moringa* + 2.5% Soybean) and WMS₅ (90% Maize + 2.5% *Moringa* + 7.5% Soybean).

shaken for 30 min. About 3 drops of ammonia solution was added to the samples and filtered. The filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H₂SO₄ and absorbance was measured using spectrophotometer at 470 nm (Okwu and Josiah, 2006)

Determination of vitamin C

The modified method of the method adopted by Awolu et al. (2013) was used. The vitamin C content of the hydrophilic extracts from the sample was determined by the spectrophotometric method using ascorbic acid as a reference compound. Exactly 10 ml of the juice sample was weighed into 10 ml of water and mixed together. 200 µl, that is, 0.2 ml of the extract was pipetted and mixed with 300 µl (0.3 ml) of 13.3% of trichloro-acetic acid (TCA) and 75 µl (0.075 ml) of dinitrophenylhydrazyl (DNPH). The mixture was incubated in water bath at 37°C for 3 h. After 3 h, 500 µl (0.5 ml) of 65% sulphuric acid was added and the absorbance was read with the spectrophotometer at 520 nm. The concentration of vitamin C was calculated as follows:

$$\frac{\text{Absorbance of standard}}{\text{Concentration of standard}} = \frac{\text{Absorption of sample}}{\text{Concentration of sample}}$$

Determination of phytochemicals

Flavonoid was determined using the procedure of Boham and Kocipai (1994). From oxalate through Day and Underwood (1986)

procedure, phytin-phosphorus was determined by the method of Wheeler and Ferrell (1971) as modified by Reddy et al. (1978). Phytic acid was calculated by multiplying Phytin-P by the factor of 3.55 (Enujiugha and Olagundoye, 2001). The tannin content was determined by the quantitative method of Makker and Goodchild (1996). Alkaloid was determined using the procedure of Harborne (1973).

Statistical analysis

All analyses were carried out in triplicates. The results obtained were subjected to analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). Means were separated using the Duncan multiple range test (DMRT) at 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Proximate composition and vitamin content of formulated Kokoro

The results of proximate composition and vitamin contents of the formulated Kokoro are shown in Figure 1. Kokoro made from WMS₀ formulation had the highest CHO (65.35%) and least crude protein (5.03%) and crude fibre (2.08%) content. The significantly ($p < 0.05$) high crude

Table 1. Phytochemical composition of formulated Kokoro.

Formulated Kokoro	Oxalate (mg/g)	Phytic acid (mg/g)	Phytic phosphate (mg/g)	Tannin (mg/g)	Alkaloid (%)	Flavonoid (%)
WMS ₀	0.22 ^d	19.86 ^d	5.67 ^d	0.014 ^c	1.57 ^c	1.92 ^d
WMS ₁	0.27 ^{cd}	24.72 ^c	6.96 ^c	0.025 ^c	2.00 ^b	3.23 ^a
WMS ₂	0.59 ^a	30.49 ^a	8.57 ^a	0.025 ^c	2.40 ^a	2.63 ^b
WMS ₃	0.36 ^b	30.32 ^a	7.31 ^b	0.029 ^a	2.00 ^b	1.95 ^{cd}
WMS ₄	0.32 ^{bc}	20.60 ^d	5.80 ^d	0.027 ^b	2.00 ^b	1.99 ^c
WMS ₅	0.36 ^b	25.98 ^b	7.31 ^b	0.017 ^d	2.40 ^a	1.94 ^{cd}

Values not followed by the same letter in the same column are significantly different ($P < 0.05$). WMS₀ (100% Maize flour), WMS₁ (90% Maize + 10% Moringa + 0% Soybean), WMS₂ (90% Maize + 0% Moringa + 10% Soybean), WMS₃ (90% Maize + 5% Moringa + 5% Soybean), WMS₄ (90% Maize + 7.5% Moringa + 2.5% Soybean), and WMS₅ (90% Maize + 2.5% Moringa + 7.5% Soybean).

carbohydrate and least crude protein found in Kokoro from WMS₀ is consistent with the study of Omueti et al. (1992) and Miracle (1997). This study showed cereal based product to contain primarily carbohydrate and to be low in crude protein. Increased substitution of soybean and moringa flour for maize flour in the formulated Kokoro increased crude protein content and decreased carbohydrate content.

Kokoro (WMS₂) had the highest moisture (11.20%) and crude protein (10.17%). Kokoro (WMS₁) showed no significant ($P > 0.05$) difference with WMS₂ with regards to moisture content. The highest moisture and crude protein content observed in Kokoro (WMS₂) was due to defatting effect on soybean flour. Defatting has been reported to increase flour water absorption capacity (Gonzalez-Agramon and Serna-Saldivar, 1988; Ogunsina et al., 2010) and crude protein (Alobo et al., 2009; Uzor-Peters et al., 2008). Serna-Saldivar et al. (1988) reported 35% more crude protein in wheat flour fortified with 11.1% defatted soybean meal (SBM).

The highest crude fibre (7.71%) content was recorded in WMS₁. The highest crude fibre recorded in Kokoro (WMS₁) is consistent with the study of Sanford (2000) and Oduro et al. (2008) that showed dried moringa leaf to contain high crude fibre concentration. Increase in substitution with dried moringa leaf powder from 0 to 15% has been reported to result in increase in dietary fibre (Dachana et al., 2010). Potential health benefits of dietary fibre have been documented in relation to the prevention of cardiovascular disease (Bazzano et al., 2003).

The highest fat content (24.67%) was obtained in WMS₅. The fat content in WMS₁ did not show significant difference ($p < 0.05$) from those obtained in WMS₄ and WMS₅. Substituting white maize flour with defatted soybean flour in the formulation of Kokoro at WMS₃, WMS₅ and 10% level of defatted soybean flour substitution (WMS₂) significantly ($p < 0.05$) increased Kokoro fat content. This is consistent with the study of Ogunsina et al. (2010) revealing the ability of defatting of soybean in increasing fat absorption capacity of flour.

The highest value of vitamin A, B and C recorded in

formulated Kokoro (WMS₁) showed the ability of moringa leaf in improving vitamin composition of Kokoro when substituted for maize in its formulation. This is in agreement with earlier study on moringa leaf that showed it to be substantially rich in vitamin A, B and C (ascorbic acid) (Ramachandran et al., 1980; Sanford, 2000; Anwar et al., 2007). The ascorbic acid acts as antioxidant and hence enhance the shelf life of fat containing food (Dillard and German, 2000; Siddhuraju and Becker, 2003).

Though vitamin B₃ (niacin and niacinamide) has been reported to possess antioxidant properties (Gliszczynska-Swiglo, 2006), and is therefore able to protect the body against free radical induced degenerative diseases due to its antioxidant properties. Dried moringa leaf has been reported to contain seven times more of the vitamin C found in orange and four times more the vitamin A obtained in carrot (Balbir, 2005; Fahey, 2005). Kokoro (WMS₀) had the least value of vitamin A, B₃ (niacin) and C. The highest value of vitamin A, B₃ (niacin) and C were recorded in Kokoro (WMS₁).

Substitution of soybean or moringa for maize in the formulated flour significantly ($p < 0.05$) improved Kokoro vitamin A, B₃ (niacin), and C. Likewise, the increase in the levels of substituting moringa for soybean in the formulated flour.

Phytochemical composition of formulated Kokoro

The results of the phytochemical composition of formulated Kokoro are presented in Table 1. The highest value for oxalate (0.59 mg/g) and phytic acid (30.49 mg/g) were recorded in Kokoro made from 10% level of defatted soybean flour substitution (WMS₂). The presence of phytate has been established in Soybean (Ronald, 2000). Phytates are organic acid, present in the bran or hulls of seeds, which blocks the uptake of essential minerals, calcium, magnesium, iron and especially zinc in the intestinal tract (Ronald, 2000) due to its ability to chelate divalent cations (Nelson et al., 1968).

Phytate content is higher in the raw white variety of the maize than the raw yellow variety (Obboh et al., 2010). It is only a long period of fermentation that can significantly reduce the phytate content. The concentration of high level of oxalate, a salt or ester of oxalic acid in Kokoro formulated with 10% level of defatted soybean flour substitution (WMS₂) was due to its presence in soybean seed. The high concentration of oxalate in Kokoro produced at all levels of moringa flour formulation may be as a result of large amounts oxalate found in the stems and leaves of *M. oleifera* (Dachana et al., 2010; Olson and Carlquist, 2001).

There were no significant differences ($p < 0.05$) between 100% white maize flour (WMS₀) formulation and WMS₄ with regards to phytic acid and phytic phosphate. Kokoro (WMS₀) had the least value for all anti-nutritional factors considered. The highest value for phytic phosphate and tannin occurred in WMS₃. The low tannin in 100% white maize flour formulated Kokoro (WMS₀) is consistent with the finding of Obboh et al. (2010) that reported low tannin content in white maize. Tannin has also been found negligible in all fractions of moringa plant (Reyes-Sánchez et al., 2006). Tannin is known to affect nutritive value of food products by forming complex with protein (both substrate and enzyme) thereby inhibiting digestion and absorption (Osuntogun et al., 1987). They also bind Fe making it unavailable (Aletor and Adeogun, 1995).

The highest values for alkaloids were recorded in WMS₅, WMS₂ and WMS₃ formulated Kokoro. There were no significant differences ($p < 0.05$) between the three formulations. The highest value for flavonoid (3.23%) was in Kokoro (WMS₁). The least value (1.92%) was revealed in (WMS₀). Flavonoid content in formulated Kokoro increases with increase in moringa flour substitution in the flour mixture. The observed rise in flavonoid content of formulated Kokoro with attendant rise in moringa flour substitution is consistent with earlier study that showed moringa leaf as a good source of natural antioxidants (Dillard and German, 2000; Siddhuraju and Becker, 2003; Anwar et al., 2005). Antioxidants are known for free radical scavenging ability (Scherer and Godoy, 2009) and therefore, neutralize free radicals that have the ability of stimulating reaction that make the cells more vulnerable to cancer causing chemicals, called carcinogen. Reddy et al. (2005) reported that the use of *M. oleifera* as source of natural antioxidant and it was found effective in controlling lipid oxidation during storage of biscuits.

Conclusions

The addition of defatted soybean and *M. oleifera* leaf flours to maize flour in the production enhanced the protein, crude fibre and vitamins contents of the snack (Kokoro). In addition, the flavonoid and alkanoids (antioxidants) contents were improved by the incorporation of deffated soybean and *M. oleifera* leaf flours. Soybean had a negative effect on the anti-

nutrients at 5% and above incorporation. Therefore, sample WMS₄ (90% maize + 7.5% moringa + 2.5% soybean) was the best formulation which guaranteed enough protein, crude fibre, vitamins and antioxidants, with considerably low anti-nutrients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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