Nutrient, Anti-Nutrient Composition and Sensory Attributes of Biscuit Made from Millet-Soy Blends

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ABSTRACT

Biscuits are commonly given to children as they grow to become toddlers, and as such efforts should be made to formulate biscuits to contribute to meeting their energy and other nutrient needs.

Objective: The potential of malted millet and soy in various blends for the development of toddler biscuits was investigated.

Materials and methods: Malted millet and soybean seeds were processed into flour. Biscuits were formulated from the flour blends using optimisation through graphical illustration of linear programming (samples A, E and D), and compared with the traditional trial and error method (samples B and C). Nutrient and anti-nutrient content and sensory attributes of the biscuit samples were evaluated using standard methods.

Results: Optimised biscuit samples had low moisture content, ranging from 4.92% to 5.42%. Protein content of the samples ranged from 11.47% - 20.39% and energy values from 422.36kcal – 473.80kcal. Sample A had highest value of 6 – carotene (418.40IU) and vitamin E (0.29ppm). Calcium content of the biscuit samples ranged from 994.13 – 1218.13% for all the samples, iron was 32.18 – 61.96 ppm and zinc was 28.41 – 52.08 ppm. Oxalate and trypsin inhibitor ranged from 736.50 mg/kg to 1002.40 mg/kg and 5.82mg/kg to 60.52 mg/kg, with phytate (1450.00mg/kg – 4650.00 mg/kg) and (1045.00mg/kg – 9080.00mg/kg) respectively. Sample A was also the most preferred in the sensory evaluation.

Conclusion: The study showed that the biscuit made from millet-soy blends contained appreciable quantities of protein, β – carotene, vitamin E, calcium and zinc. The anti-nutritional composition of the biscuits were found to be within acceptable limits.

Keywords: Toddler, Biscuits, Millet, Soybean.

INTRODUCTION

Rapid growth occurs as infants grow into toddlers resulting in increased energy and nutrient needs that can no longer be met by breast milk alone (1). At this stage, breast milk which has been established as the best source of nutrients for the infant, (1, 2) no longer meets the nutrient requirements of the child. However, complementary foods are usually introduced and by one year, an increasing amount of their diet as 'solids', which encompass a wide range of foods and drinks taken by cup, by spoon, by hand, in mixtures, combination or alone is given. The World Health Organization (WHO) recommends that infants should start receiving complementary

foods at 6 months of age in addition to breast milk, initially 2-3 times a day between 6–8 months, increasing to 3–4 times daily between 9-11 months and 12-24 months (3). WHO also recommends that finger foods also known as snacks be given to infants of 6-23 months, once or twice per day based on the child's appetite (3) to meet the recommended nutrient targets.

The nutritional needs of a child from 6 months up till the toddler age changes during this time, as the child learns to handle different foods, develops a fondness or otherwise for new tastes, and establishes a multifaceted relationship with what is eaten (1). Finger foods are usually soft, easy to grasp and mostly cereal based. Examples include biscuits, crackers some fruits and vegetables (such as banana, carrot). Biscuits as finger foods, when fed to the child, are expected to contain the nutrients needed by toddlers, including carbohydrates, proteins and fats, and micronutrients such as vitamins and minerals (5).

Cereals, when mixed with legumes for consumption gives a better nutrient profile. Millet is one of the cereals produced extensively in Nigeria. It is a good source of nutrients and can be malted to increase physicochemical accessibility and bioavailability (6,7). Malting also has the potential to modify the starch content of the cereal such that they become less viscous (8). Soybean (*Glycine max*) contains a reasonable amount of protein, minerals, vitamins and even phytochemicals such as isoflavones (9), and has good balance of amino acid patterns. This composition allows it to complement millet in providing protein to a child's diet.

Most finger foods given to children of complementary feeding age and toddlers are not nutritionally adequate for their age group (10) with many of these foods containing high levels of dietary sugar, salt and fats. Consuming such inappropriate foods have contributed to the overconsumption of discretionary calories and sodium which have been implicated in dental caries and other non-communicable diseases.

Hence, this study determined the nutrient composition of the formulated optimized millet – soy based complementary biscuit, and also evaluated its acceptability for toddlers.

MATERIALS AND METHODS Sample Collection

Soybean was purchased in an open market at Iwo, Osun State while millet was obtained from an open market at Ogbomoso, Oyo State, Nigeria.

Sample Preparation

Preparation of malted millet flour

The millet was sorted out to remove extraneous materials. It was then washed thoroughly with clean water. The millet grains were soaked separately in water (1:2 w/v) for 6 hours with the water being change at 2 hours interval. After 6 hours, the water was drained and the grains were kept in a dark cupboard to germinate. The grains were watered 2 times a day at regular intervals. After germination for 2 days (48 hours), the grains were dried in a hot air oven dryer at $60^{\circ}C$ overnight. The dried malted grains were dry

milled in a laboratory Kenwood blender and sieved with a $500\mu m$ screen sieve to obtain fine flour.

Preparation of soybean flour

Soybean was soaked in warm water for 6 hours and then de-hulled manually. The de-hulled soybean was steam cooked for 20 minutes, and sun dried. It was allowed to cool and dry- milled properly into flour using a laboratory Kenwood blender and sieved with a 500μ m screen sieve to obtain a fine flour. This was kept in a Ziploc bag for analysis.

Optimization by Linear Programming

Nutrient Optimization by linear programming using graphical representation in Microsoft Excel (11) was carried out in order to identify the combination of millet and soy to meet two nutritional constraints, energy then protein, at the lowest possible cost. Hence, 746 kcal of energy at 9 months of age, was used as (12) estimate of nutritional requirements during complementary feeding period.

To determine the combination of millet and soy that will provide the required amount of energy, i.e., 746 Kcal, the following equation was used

$$\frac{E_{mf}.X_{mf}}{100} + \frac{E_{sf}.X_{sf}}{100} = 746$$

Where $X_{mf,} X_{sf,} E_{mf,}$ and E_{sf} represent the weight (grams) and the energy content (kcal/100g) of millet flour and soy flour respectively. These combinations were represented graphically with all combinations below the line providing less than the required energy. The combinations of millet and soy obtained were used to prepare flour blends for biscuit production.

Flour blends formulation

Two combinations of millet and soy that could provide the required amount of energy from nutrient optimization by linear programming, using graphical representation (Fig 1.) in Microsoft Excel, were picked [100mf:86sf (sample A) and 200mf:5sf (sample E)], one from optimization using Microsoft excel [100mf:60sf (sample D)], with another two from the traditional trial and error method (50mf:50sf (sample B) and 40mf:60sf (sample C)] and then a reference sample [100wf (sample F)]. The malted millet and soy flour were weighed respectively and blended together to obtain a composite malted millet-soy composite flour used in baking biscuits.



Fig 1: Graph showing the combinations of millet and soy for energy constraints

Production of biscuit

Biscuit was produced using the conventional creaming method. Fat and sugar were creamed together till the mixture was fluffy. After creaming, flour, baking powder and milk with egg yolk as well as a pinch of salt were added and mixed until batter was uniformly mixed. The batter was manually kneaded to ensure uniformity. It was then transferred to a clean tray and gently rolled into a sheath using a roller. The batter sheath was cut into shapes using cookie cutter and placed into a greased pan. This was then baked in the oven at 200°C for 15min. The baked biscuits were left to cool before packaging in Ziploc bags for analysis (Plate 1).

Proximate Composition Determination

The proximate composition of the biscuit samples was determined according to standard methods described by AOAC (13). Metabolizable energy was calculated using the value for ether extract, crude protein and carbohydrate in kilocalories. Metabolizable energy was calculated by multiplying the nutrient content by factors 4, 4 and 9 for carbohydrate protein and fat respectively in kilocalories.

Vitamin Composition

 β – Carotene and vitamin E were determined using the method of (13). 1 gram of each samples

was weighed into glass sample bottles. 10ml of N-Hexane was added and shaken for about 1hour. This was allowed to stay overnight in the dark. The mixture was then filtered with Whatman paper no 1 and left in the fume hood for the hexane to evaporate. 5 mls of HPLC grade methanol was added and the sample injected into HPLC. The reading was taken for β – Carotene at 325nm and vitamin E at 290nm.

Determination of Minerals

Calcium, zinc and iron were analyzed by the AOAC method (13). The biscuit samples were ashed in a muffle furnace at 550 °C for 5 hrs. The mineral extract was obtained by digesting the ash with 6N HCl, 5 ml of 6N HCl was added to the ash and filtered, the extract (filterate) was made up to 50 ml with distilled water. Iron and zinc were d etermined by atomic-absorption spectrophotometry (PG 990, PG Instrument Ltd, UK) and Calcium was determined by flame photometer (Jenway PFP7, Jenway Scientific instruments, UK). The analyses were done in triplicates. The results were obtained after three readings and expressed in mg/Kg.

Sensory Evaluation

The sensory evaluation was performed on the millet-soy biscuit prepared from various combinations using 12 panellists comprising of

nursing mothers who are experienced in complementary feeding. The samples were kept in Ziploc bags at room temperature. Panellists rated the biscuit samples for colour (very dark brown to very light brown), texture – hand feel (softness to hardness), smoothness - mouth feel (smooth or gritty), aroma, taste and overall acceptability using 1-5 hedonic scale where 1 represents dislike extremely, 2 – dislike, 3 like moderately, 4–like and 5 like extremely.

Statistical Analysis

The data generated were analyzed using analysis of variance (ANOVA), and means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability ($p \le 0.05$). The Statistical Package for Social Scientist (SPSS) software, version 20.0 was used for the analysis.

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of the biscuit samples is presented in Table 1. Moisture content (MC) of all the samples ranged from 5.10 - 11.98%. There was no significant difference in the MC of the millet-soy samples at $p \le 0.05$, but that of the reference sample (100% wheat biscuit) was significantly higher than those of all the millet-soy samples. The optimized samples had lower MC than samples made using the traditional trial and error method. This indicates better stability in the storage shelf life, as studies have revealed that low moisture content in food products will prevent the growth of mould, reduce moisture-dependent biochemical reactions (14, 15) and therefore enhance the storage stability of the biscuits.

Protein content of the samples were significantly different as the soy flour contributed greatly to the biscuit formulation except for sample E that had the lowest protein content and was not significantly different at $p \le 0.05$ from the reference sample (sample F). The protein values of millet – soy samples indicates that they can contribute to protein requirements during the complementary feeding period.

The fat content of the millet – soy biscuit samples were higher than that of the reference sample which had the lowest mean. This may allow for the absorption of fat-soluble vitamins, provide essential fatty acids such as omega-3 and omega-6 polyunsaturated fatty acids (PUFA) required for normal brain development, healthy skin and resistance to infection and disease in in children of complementary feeding age (16). There was significant difference at $p \le 0.05$ in the mean of the samples. The optimized samples had low fat content as compared to those from the trial and error method though they all are within the percentage suggested in literature (17). This attribute is favourable since the possibility of excessive fat intake (and its implications) could be avoided.

There was significant difference at $p \le 0.05$ in the mean of the energy values of the biscuit samples. The energy values were higher in the millet – soy samples than the reference sample, sample F, which had the lowest. This can contribute to the estimated energy requirement by (12) for infant and young children who are required to have 682 kcal/day, 830 kcal/day and 1,092kcal/day for 6-8 month, 9-11 month and 12- 23 month child respectively which is needed for optimum growth and development of the child.

Comparatively, the fibre content of the biscuit samples was low with the reference sample (sample F) having the lowest mean value for fibre. This indicates that the fibre content might not form a fibre-mineral complex since fibre has been found to bind minerals which might not be digestible by human (18). There was significant difference in the fibre, ash and carbohydrate content of the sample. The high carbohydrate contents observed in this study are nutritionally desirable as children require energy to carry out their rigorous physical and physiological activities as growth continues (19).

Vitamin and Mineral Composition

The mean value for β – Carotene ranged from 25.1 – 418.4 IU for all the biscuit samples with significant difference at $p \le 0.05$. In the millet-soy biscuit, sample A had the highest mean value and sample B had the lowest, and same mean value with the reference sample (sample F). This indicates that the addition of soy flour in high proportion contributed greatly to β – Carotene content of the biscuit. Vitamin E values ranged from 0.09 - 11.5 ppm for all samples at $p \le 0.05$. Vitamin E, a fat-soluble vitamin, protects vitamin A and essential fatty acids in the body and prevents the breakdown of tissues. There was no significant difference in the vitamin E content of the biscuit samples. Samples A and C had higher vitamin E content out of all the millet-soy biscuit samples.

Table 1 shows the mineral composition of the biscuit samples. There was no significant difference at $p \le 0.05$ in the calcium value of all the samples except in sample E that had the lowest calcium content of 994.13%. Iron content

of the biscuit samples ranged from 32.18-61.96 ppm. The optimized samples, A, D, and E had a relatively higher iron content, 61.96, 51.15, and 54.34ppm respectively than the iron content of the arbitrary ratios, B and C which was 45.31 and 51.15, respectively. The optimized samples had a relatively higher iron content than those from the trial and error method. The zinc content of the samples ranged from 28.41 - 52.08 ppm with significant differences at $p \le 0.05$. The optimized

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1 – 200 millet flour: 5 soy flour, F – 100 refined wheat flour

samples had relatively high zinc content than biscuit samples from the trial and error method. The optimized samples A, D and E had 52.08ppm, 39.96ppm and 46.31ppm respectively as against 28.41ppm and 36.78ppm of samples B and C. The high value in the zinc and iron content of the optimized samples could be due to the higher content of millet in the biscuit composition.

Samples MC (%)	DM (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	(%)	ME (Kcal)	(%)	(ppm)
A 5.10	。 94.90 ₀	20.39 _°	23.59 _b	2.73 _{bc}	3.71 _°	44.48 _{cd}	459.82 _{ab}	1149.23 _°	61.96
B 6.80	。 93.20₀	16.81 _b	22.51 _{bc}	3.16 _b	3.59 _°	47.13 _c	448.37 _b	1142.16 _a	45.31
C 6.47	, 93.95 ₀	17.86 _b	27.72 _°	2.45 _c	3.81 _°	41.70 _d	473.80 _°	1175.54 _a	50.49
D 5.42	。 94.58α	17.42 _b	21.52 _c	4.32 _a	3.76 _a	47.57 _c	444.09 _b	1185.41 _a	51.15
E 4.92	, 95.08 _a	11.47 _c	18.81 _d	2.61 _c	2.90 _b	59.30 _°	446.74 _b	994.13 _b	
F 11.9	8 _a 88.02 _b	12.17 _c	17.82 _d	1.28_{d}	2.13 _c		422.36 _c	0.0	54.34

Anti-Nutrient Composition

Anti-nutrients reduce the bioavailability and bioaccessibility of food minerals through interference during absorption (18, 20) which can contribute to the high prevalence of mineral deficiencies (21). The result of the anti-nutrients in the biscuit samples is presented in Table 2. The mean value for oxalate and trypsin inhibitor ranged from 736.50 mg/kg - 1002.40 mg/kg and 5.82 – 60.52 mg/kg respectively with the reference sample, (Sample F) having the lowest mean value. Oxalate and trypsin inhibitor in the optimized samples were significantly different based on the combination with sample A having the lowest trypsin inhibitor value, 43.80mg/kg and sample E having the lowest oxalate content, 849.30mg/kg. Oxalate binds with calcium and iron and causes these minerals to crystalize and thus the body cannot properly utilize the calcium and iron (15).

Phytate and tannin content of the biscuit samples ranged from 1450.00mg/kg – 4650.00 mg/kg and 1045.00mg/kg - 9080.00mg/kg respectively with sample D having the highest mean value in both. The optimized samples generally had high anti-nutrient content as compared to those of the trial and error method. High level of phytate limits the bioavailability and hence utilization of minerals specifically calcium, magnesium, iron and manganese by forming insoluble compounds that are indigestible while tannins are antinutritional factors known to bind protein including digestive enzymes leading to poor protein digestibility (15).

Interrelationship between Calcium, Zinc, Phytate and Oxalate

The molar ratios for phytate, oxalate, zinc, and calcium were calculated as shown in Table 3 to determine the estimated bioavailability of these minerals in the biscuit samples. Antinutritional components in plant foods such as tannins, phytate, oxalate and polyphenols can reduce bioavailability of minerals such as Zinc, Calcium and Iron by forming insoluble complexes with these minerals (22, 23). Phytate-mineral molar ratios are used to calculate phytate inhibitory effect on mineral bioavailability (24). Phytate:Calcium molar ratio >0.24 will impair calcium absorption (25, 26) while Phytate: Iron molar ratio >0.15 will significantly decrease the iron absorption (27, 28). A report (29) indicated that zinc absorption is greatly reduced and results in negative zinc balance when phytate: zinc molar ratio is > 15. In this study Phy:Ca ratio of all the biscuit samples were lower than the critical value (< 0.24) except sample D which was 0.24. Phy:Zn ratio of Sample A was the lowest (2.75) out of all the biscuit samples implying that it will high Zn bioavailability. According to (30), based on WHO cut offs, Phy:Zn <5 has high Zn bioavailability, >5-10 moderate Zn bioavailablity, 11-15: low Zn bioavailability. Samples C, E & F have adequate Zn bioavailability while Sample D may have low Zn availability. However due to the dietary effect of Calcium on Zn absorption in the presence of phytates (31, 32) stated that the {Phy}{Ca}x{Phy}/{Zn} millimolar ratio with critical value of <0.50mol/kg is a better predictor of Zn bioavailability in foods. From our results all the biscuit samples had molar ratio below the critical value (0.08-0.31) indicating that the Zn

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So	amples	Oxalate (mg/kg)	Tannin (mg/kg)	Trypsin inhibitor (mg/kg)	Phytate (mg/kg)
A		872.95 _{bc}	4540.00 _c	43.80 _{bc}	1450.00 _d
В		830.30 _c	3305.00 _d	40.26 _c	1550.00 _d
С		909.24 _b	3285.00 _d	43.81 _{bc}	3100.00 _c
D		1002.40 _a	9080.00 _a	60.52 _{ab}	4650.00 _α
E		849.30 _c	5070.00 _b	68.15 _°	3700.00 _b
F		736.50 _d	1045.00 _e	5.82 _d	3900.00 _b

Mean values with the same subscript in the same column are not significantly different at ($p \le 0.05$). **Key: A** - 100 millet flour: 86 soy flour, **B** – 50 millet flour: 50 soy flour, **C** – 40 millet flour: 60 soy flour, **D** – 100 millet flour: 60 soy flour, **E** – 200 millet flour: 5 soy flour, **F** – 100 refined wheat flour.

Samples	{Ca} ¹	{Fe} ²	{Zn}³	{Phy}⁴	{Ox}5	{Phy}:{Ca } [¢]	{Phy}:{Zn} 7	{Phy}:{Fe} °	{Ca}:{Phy }/{Zn}°	{Ox}:{Ca}	{Ox}:{Fe} יי
Sample A	28.67	1.11	0.80	2.20	6.81	0.08	2.75	1.98	0.08	0.24	6.14
Sample B	28.50	0.81	0.43	2.35	6.48	0.08	5.47	2.90	0.16	0.23	8.00
Sample C	29.33	0.90	0.56	4.70	7.10	0.16	8.39	5.22	0.24	0.24	7.89
Sample D	29.58	0.92	0.61	7.05	7.83	0.24	11.56	7.66	0.34	0.26	8.51
Sample E	24.80	0.97	0.71	5.61	6.63	0.23	7.90	5.78	0.19	0.22	6.84
Sample F	30.39	0.58	0.59	5.91	5.75	0.19	10.02	10.19	0.31	0.19	9.91
Critical	value					>0.24	>15	>0.15	>0.50 ¹²	<u>×</u>	<u>×</u>

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content in these biscuit samples will be bioavailable and their phytate contents will not impair Zn absorption with sample A having the highest Zn bioavailability (33, 34).

All the biscuit samples had Ox:Ca less than the critical value of 1. This indicated that the oxalate content of the biscuit samples is not expected to affect the bioavailablity of the Calcium. However, the Phy:Fe of the biscuit samples showed that the molar ratios were higher than the critical value of 1 (<1), hence phytate can affect the bioavailablity of Iron in these biscuit samples.

Sensory Evaluation

The sensory quality of the biscuit samples made from millet and soy evaluated shows significant difference at $p \le 0.05$ in the quality attributes (Table 4). One of the organoleptic characteristics that influence consumer acceptance is the colour. Samples D and C were rated brown in colour, while E, A, and B were rated light brown as against the very light brown colour of the reference sample. The brown colour indicates that the biscuits are well baked. Similar results were obtained in a similar probe (5).

The smoothness (mouth feel) attribute of both the optimized and trial and error composed samples were rated slightly gritty except for sample D which was rated gritty as compared to the smooth mouth feel of the reference sample. This implies that the millet-soy samples can easily be broken and soft enough to give a child of complementary feeding age. The aroma of samples A and E were ranked high respectively next to that of the reference sample by the panelists. In terms of taste, sample A was more preferred out of the millet-soy biscuit and ranked high next to the reference sample.

Generally sample A, of the optimized samples was rated higher and most accepted by the panellists and more preferred along with the reference sample followed by samples E and B.

CONCLUSION

The investigation carried out on the potential of millet-soy biscuit for toddlers showed that the optimized sample, sample A, which was most preferred of the millet - soy based biscuit samples, ranked next to the reference sample, sample F (100% wheat flour), in terms of sensory attributes. Sample A also had favourable compositional properties such as low moisture content, high fat and protein content. It also had the highest mean value for the iron and zinc content out of all the samples and lowest antinutrient content amongst the optimized samples. Its nutrient-antinutrient ratio is generally lower than the suggested critical value which implies it has bioavailable calcium and zinc which can be consumed by infants and toddlers with little or no interference in their absorption. Therefore, this study asserts that complementary snacks (biscuits) can be produced and tailored towards toddlers using millet and soybean, to meet their dietary needs and ensure adequate nutrition for both physical and cognitive development.

Samples	Colour	Texture	Taste	Aroma	Smoothness	General Acceptability
Α	3.56 _d	2.89 _b	4.11 _b	3.67₅	2.89 _b	4.00 _b
В	3.78 _c	2.67 _c	3.22 _c	2.78_{d}	2.33c	3.11 _d
с	4.33₅	2.67 _c	3.22 _c	2.78_{d}	2.00 _e	2.56 _e
D	4.44 _a	3.11 _a	2.78 _e	2.56 _e	1.78 _f	2.56 _e
E	3.33 _e	1.78 _e	3.00 _d	3.33 _c	2.22 _d	3.56 _c
F	1.22 _f	2.00 _d	4.44 _a	4.78 _a	3.11 _a	4.92 _a

 Table 4: Sensory Evaluation of the Biscuit Samples

Mean values with the same subscript across the same row are not significantly different at ($p \le 0.05$). **Key: A** - 100 millet flour: 86 soy flour, **B** – 50 millet flour: 50 soy flour, **C** – 40 millet flour: 60 soy flour, **D** – 100 millet flour: 60 soy flour, **E** – 200 millet flour: 5 soy flour, **F** – 100 refined wheat flour









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