Nutritional Properties and Toxicological Assessment of High Nutrient Biscuit Developed from Blends of Some Cereals and Legume

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Abstract

This study sought to evaluate the nutritional and antioxidant properties of high nutrient biscuits from some cereals and legume. Toxicological investigations were also carried out on the biscuit developed alongside a commercial biscuit using some selected parameters. Proximate, mineral and vitamin composition of the biscuit were determined. Rats were fed with the high nutrient biscuits, commercial biscuit and normal rat feed for twenty-eight (28) days and were sacrificed by cervical dislocation. The Liver, heart and kidney tissues were analysed for liver (Alanine amino transferase [ALT], Alkaline Phosphatase [ALP], Aspartate amino transferase [AST], Albumin [ALB], Bilirubin [BIL] Total protein (TP)) and kidney (creatinine [CREA] and urea) enzyme and protein levels, including glutathione (GSH), glutathione peroxidase (GPX), glutathione –S- transferase, catalase, superoxide dismutase (SOD) activities, and malondialdehyde (MDA) levels as well as lipid profiles which cover for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). Feeding on the biscuits let to the significant (P<0.05) decrease in AST, ALT, ALP, BIL, urea and creatinine levels. However, relative to the control, the high nutrient biscuit (BRB) had lower concentration of these enzymes and proteins when compared to the conventional biscuit (ARB) and the control (BRC) although there was increase in the ALB and TP content of the BRB group compared to ARB and BRC. Furthermore BRB had significant reduction in the TC, TG, LDL, and HDL concentration followed by the ARB when compared to the BRC groups. Moreover there was significant increase in the levels of the antioxidant enzymes and decrease in malondialdehyde production in the liver, heart and kidney of BRB when compared to other groups. This result indicates high nutritional properties and antioxidant potential of the biscuits. Therefore the high nutrient biscuit could be used as functional food and an adjunct dietary therapy for malnourished children.

Keywords: Biscuit; Nutrition; Anti-oxidant; Malnourish; Children

Introduction

Malnutrition is a major health problem common to underdeveloped and developing countries of the world. It is a major cause of death and accounts for fifty percent deaths in children less than five years [1]. Recent reports have shown that 43 percent of children are stunted all over the world due to poverty and inaccessibility to good food. Moreover stunting is commonly used as an index for long-term chronic nutritional deficiency [2]. Nutritional deficiency has been linked with high free radical generation [3]. Free radicals are capable of inducing oxidative stress which has been implicated in the development and progression of some diseases such as diabetes, cardiovascular and neurodegenerative diseases [4,5].

One of the Major interventions for the treatment and/or management of malnutrition is fortification of food with essential nutrients [6]. Many food products such as bread, biscuit, snacks and confectioneries are vehicles used to deliver major nutrients to the body [7]. Development of fortified biscuit is widely acceptable since it is a good vehicle of supplementation with proteins, carbohydrate, minerals and fats including phytochemicals. This major characteristic is due to the fact that biscuits are ready to eat, popular, easily accessible, cheap, has high nutrient density and long shelf life. Biscuit are usually produced with different ingredients such as flour, shortening (margarine), sweeteners (sugar) and milk. The type of cereals and other constituents present in a biscuit determines its nutritional quality and medicinal properties.

Previous reports have shown that cereals and legumes such as sorghum, maize and soybean contain appreciable levels of micro and macro nutrients including phytochemicals which can help to combat malnutrition as well as prevent oxidative stress and diseases associated with malnutrition [8,9]. This study sought to evaluate the nutritional properties of high nutrient biscuit developed from sorghum, maize and soybean flour as well as assessing its toxicological effects.

Materials and Methods

Materials

Soybean, sorghum, maize, wheatflour, sugar, salt, fat, Sodium bicarbonate (baking powder), lecithin and milk were purchased from the main market in Mushin Lagos State Nigeria.

Methods

Production of biscuit: Biscuits were produced at laboratory scale using the method of Ayo [10] with a slight modification.

Determination of nutritional composition: Nutrition composition of the biscuit was determined using established methods. Proximate and mineral composition was determined using the method of AOAC [11]. Vitamin analysis was also carried via high performance liquid chromatography (HPLC) using the method of AOAC [11].

Sensory evaluation: Sensory evaluation was conducted on the developed biscuit at laboratory scale according to the method described [12]. It was compared to readily available commercial biscuit. They were given the reference codes HNB and CB for the developed and

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commercial biscuit respectively. The coded samples were presented to a 10-men panelist to evaluate for the attributes: colour/appearance, taste, after taste, mouth feel, crispiness, crunchiness texture, flavour, aroma, and overall acceptability. Scores were given to the scales: (1) extremely unacceptable (2) very unacceptable (3) moderately unacceptable (4) slightly unacceptable (5) neither acceptable nor unacceptable (6) slightly acceptable (7) moderately acceptable (8) very acceptable and (9) extremely acceptable.

Animal experiment: Male albino rats (25) with mean weight 95 ± 5.32 g and mean age 3-4 weeks were divided into three groups BRC CB and HNB and housed in rat cages. They were allowed access to water and feed ad libitum for them to acclimatize to laboratory conditions. After this period, the control animals (BRC) were continues on the commercial feed uninterrupted, while CB animals were placed on commercial biscuits and group C on the baked soy biscuit. The animals were fed for 28 days with the appropriate feeds and water ad libitum. The animals were weighed immediately before commencement of the feeding experiment and afterwards at a week intervals for the remaining days the was carried out.

Determination of hematological parameters: Hematology profile, which covers hemoglobin level (HGB), packed cell volume (PCV), red blood cell (RBC) count, white blood cell count (WBC), platelets (PLT) was determined using a Synchron CX5 autoanalyzer according to the manufacturer’s protocol.

Determination of serum biochemistry (liver and renal function enzymes, lipid profile): Blood serum was used for the evaluation of biochemical parameters, including urea, creatinine, total bilirubin, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, using commercial kits from Randox Laboratories, UK, according to the manufacturer’s protocol. Serum’s total cholesterol, triglyceride, and high-density lipoprotein (HDL) levels were also measured via enzymatic colorimetric method using Randox kits [13].

Determination of oxidative stress parameters: Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR). Catalase (CAT) activity was estimated by measuring the rate of decomposition of H2O2 using the method of Aebi [14]. The level of superoxide dismutase (SOD) activity was determined [15]. While the method of Ellman [16] was adopted in estimating the activity of reduced glutathione (GSH).

Data analysis: Results were pooled and statistical significance was established using one-way analysis of variance, and data were reported as mean ± standard error. Significant difference was established at p<0.05. Statistical analyses were carried out using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL).

Results and Discussion

There are indications that foods and nutrients contribute to normal functioning of the body. Inclusion of cereals and legumes with bioactive compounds and nutraceuticals in human nutrition has been shown to alleviate malnutrition and its associated diseases such as neurodegenerative diseases, diabetes, diverticulosis and cardiovascular diseases [17]. The proximate composition of the high nutrient density biscuit as shown in Table 1 revealed appreciable amounts of carbohydrate (59.34%), protein (17.50%) and high calories (440.7 kcal). Table 2 shows the mineral composition of the high nutrient density biscuit. Minerals such as iron (5.9 mg/g), Magnesium (39.0 mg/g), Phosphorus (145.1 mg/g), Zinc (1.2 mg/g), Selenium (4.9 mg/g) and Calcium (191.0 mg/g). Furthermore, Table 3 shows the presence of vitamin B1 (0.12 mg/g), B2 (0.10 mg/g), B6 (1.50 mg/g), B12 (0.10 mg/g), B12 (40.10mg/g) and D (1.30 mg/g). Furthermore, sensory characteristics of the laboratory scale developed biscuit and a commercial biscuit were done using parameters such as color/appearance, taste, after taste, mouthfeel, texture, crispiness, crunchiness, flavour and overall acceptability. Similar result was obtained in both biscuit samples over a period of five months as shown in Table 4. There was decrease in the sensory characteristics over the months.

The results of the haematological indices are shown in Table 5. There was no significant (P<0.05) difference in all the hematological parameters that were determined in the control, CB and HNB groups. This shows that feeding on the biscuit does not pose any risk of diseases since increase or decrease in any of these parameters indicate

| Parameters            | HNB       |
|-----------------------|-----------|
| Moisture (%)          | 5.9 ± 0.35|
| Protein (%)           | 14.9 ± 0.25|
| Crude fat (%)         | 17.11 ± 0.23 |
| Ash (%)               | 2.04 ± 0.18 |
| CCOhydrate (%)        | 59.34 ± 9.7 |
| Energy (K cal)        | 440.7 ± 2.25 |

HNB - high nutrient biscuit developed from soybean. Values represent means of triplicate readings.

| Minerals       | HNB |
|----------------|-----|
| Iron           | 5.9 ± 0.06 |
| Magnesium      | 39.0 ± 0.25 |
| Phosphorous    | 145.1 ± 2.2 |
| Zinc           | 1.2 ± 0.05 |
| Selenium       | 4.9 ± 0.12 |
| Calcium        | 191.0 ± 1.2 |

HNB - high nutrient biscuit. Values represent means of triplicate readings.

| Vitamins      | HNB            |
|---------------|----------------|
| B1            | 0.12 ± 0.0002  |
| B2            | 0.10 ± 0.0001  |
| B3            | 1.50 ± 0.0003  |
| B4            | 0.10 ± 0.0005  |
| D             | 1.30 ± 0.0003  |
| B12           | 40.10 ± 2.14   |

HNB - high nutrient biscuit. Values represent means of triplicate readings.

| Biscuit attributes | HNB            |
|--------------------|----------------|
| Colour/Appearance  | 7.4 ± 0.67     |
| Taste              | 7.2 ± 0.69     |
| After taste        | 6.54 ± 0.55    |
| Mouthfeel          | 6.26 ± 0.54    |
| Texture            | 7.02 ± 0.78    |
| Crispiness         | 6.50 ± 0.67    |
| Crunchiness        | 6.44 ± 0.62    |
| Flavour            | 6.96 ± 0.81    |
| Overall acceptability | 6.72        |

HNB - high nutrient biscuit. Note: Values = mean ± SD; n = 10.

| Table 4: Sensory attributes of developed biscuits. |
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Values represent means of triplicate readings. Values with the same letter along the rows are not significantly (P<0.05) different. 

Table 1: Lipid profile of the experimental groups.

| Parameters | Control (BCR) | CB | HNB |
|------------|---------------|----|-----|
| TC (mg/dL) | 154.4 ± 6.9* | 160.0 ± 8.0* | 164.2 ± 7.5* |
| TG (mg/dL) | 89.2 ± 2.3* | 95.0 ± 2.8* | 100.0 ± 2.5* |
| HDL (mg/dL) | 37.0 ± 3.0* | 38.0 ± 3.1* | 39.0 ± 3.2* |
| LDL (mg/dL) | 81.0 ± 2.5* | 85.0 ± 2.7* | 88.0 ± 2.9* |

Values represent means of triplicate readings. Values with the same letter along the rows are not significantly different at P>0.05.

Table 2: Hematological profile of the experimental groups.

| Parameters | BRC | CB | HNB |
|------------|-----|----|-----|
| RBC (x1012/L) | 7.3 ± 0.1* | 7.4 ± 0.2* | 7.6 ± 0.3* |
| Hgb (g/dL) | 14.6 ± 1.2* | 15.0 ± 1.3* | 15.4 ± 1.4* |
| PCV (%) | 42.1 ± 2.1* | 42.5 ± 2.2* | 43.0 ± 2.3* |
| MCHC (g/dL) | 32.1 ± 1.2* | 32.5 ± 1.3* | 33.0 ± 1.4* |

Parameters Control CB HNB

ALT (µL) 40.56 ± 13.2* 21.4 ± 8.63* 20.63 ± 10.2* 
AST (µL) 210.01 ± 15.8* 224.8 ± 18.5* 232.83 ± 16.4* 
ALP (µL) 42.86 ± 10.3* 66.5 ± 5.32* 65.2 ± 8.53* 
ALB (g/L) 16.76 ± 4.21* 21.4 ± 3.11* 20.63 ± 2.91* 
TP (g/L) 31.66 ± 10.5* 45.8 ± 10.5* 38.42 ± 9.7* 
BIL (µmol/L) 0.36 ± 0.10* 1.56 ± 0.50* 0.46 ± 0.15* 

Parameters Control | CB | HNB |
|-------------------|----|-----|
| CREA (µmol/L) 29.93 ± 6.5* | 21.72 ± 3.9* | 15.97 ± 2.5* |
| Urea (mmol/L) 1.66 ± 0.8* | 0.93 ± 0.41* | 1.36 ± 0.6* |

Table 3: Blood chemistry parameter for kidney function.

Figure 1: Lipid profile of the experimental groups. TC= Total cholesterol, TG= Triglyceride, LDL= Low density lipoprotein, HDL= high density lipoprotein, BCR= Control group, CB= rats fed with commercial biscuit, HNB= Rats fed high nutrient biscuit.
activities than CB. Higher antioxidant enzyme activities will reduce oxidative stress which has been associated with severe malnutrition.

Malondialdehyde (MDA) is one of the products of lipid peroxidation. Its presence in tissues indicates oxidative stress. Results from this study showed that feeding on the biscuit reduced MDA levels in the heart, liver and kidney. However, MDA levels in HNB were significantly lower than CB and BRC (Figure 5). Nwozo and Oyinloye also reported decrease in MDA levels in rats’ liver which was caused by aqueous extract of *Afromomum melegueta* [28].

**Conclusion**

The nutritional properties, high antioxidant activity and protective effects of the high nutrient biscuit on liver and kidney suggests its potential as functional food and adjunct therapy for the management and/or treatment of malnutrition and malnutrition-induced oxidative stress.

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