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# IN VITRO PROTEIN DIGESTIBILITY AND MINERAL BIOAVAILABILITY OF COOKIES PRODUCED FORM WHEAT - FLUTED PUMPKIN (TELFERIA OCCIDENTALIS HOOK) FLOUR BLENDS

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Cookies were made from wheat and fluted pumpkin (Telferia occidentialis hook) seed flour blends at different levels of substitution and analyzedfor proximate composition and sensory properties. The most acceptable sample (15%) was used to determined minerals antinutrints and in-vitro protein digestibility as an index of bioavailability. The protein levels (0%-20%) in cookie ranged from 6.125%-7.70% of fluted pumpkin flour, whereas carbohydrate contents reduced from 44.6% to 34.02%. Samples also showed acceptability up to 15% level substitution for cookies. 3.61, 3.33, 3.50 and 3.0 for 0%, 5%, 10% and 15% levels of substitution with fluted pumpkin flour respectively. Calcium and iron content of cookies made from the composite flour increased significantly (15.8-24.5 mg) and (0.14-4.6 mg) respectively. There was a significant reduction in phytic acid content (6.16-2.93%) for cookies. Tannic acid did not show any significant increase with increase in the levels of substitution with fluted pumpkin flour cookies (0.210-0.22). Digestibility levels of products made from composite flour increased significantly in the cookies (18.40-92%). In conclusion, the products made from the composite flour contained more protein, improving the nutrient properties of the flour hence increasing, it's usage in the food industry. Adequate protein digestibility increases the use of fluted pumpkin flour for supplementation.

***Keywords:*** Cookies, Protein levels, Fluted pumpkin, Flour blends

## INTRODUCTION

Recent estimates from the FAO indicates that 840 million people in the world do not receive enough energy protein and micro-nutrients (FAO, 2003). The overhauling majority of these people about 799 million line in developing countries. This has necessitated contemporary research effort geared towards the study of the food properties and potential utilization of protein and other nutrients from locally available food crops especially from underutilized or relatively neglected high protein oilfeeds and legumes Achi (2005).

One of the locally available under exploited but a potential high protein food source in Nigeria is fluted pumpkin (Telferia occidentials Hook) seed. In addition to its importance as an oilseed (54% fat), it is a valuable source of protein (27%) with a high content of micronutrient, Longe *et al.* (1983). However , the usefulness of fluted pumpkin seed as a protein source for human food is limited by the presence of anti-nutrients, particularly phytic acid (Akwaowo *et al.*, 2000), which has been shown to lower the bioavailability of minerals in humans and to inhibit the digestibility of plant proteins (Lopez *et al.*, 2002).

Studies on bioavailability of protein by previous worker (Drake, 2007), have shown that processing methods such as heat treatments, fermentation or germination reduce phytic content and enhance HCL extractability and

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digestibility of protein in cereals, Legumes and oilseed.

Confertionary products such as cookies are usually patronized by children and adults, therefore are important local snacks which would need to be supplemented with high protein oilseeds, such as the fluted pumpkin seed.

The purpose of this study was to evaluate the sensory and chemical composition of two confectionery products cookies made from wheat – fluted pumpkin flour blends and the determination of in-vitro protein digestibility of the products.

## MATERIALS AND METHODS

Fluted pumpkin (Telfaria occidnetialis hook) seed was obtained form the oil mill markets in Port Harcourt, Nigeria for this study. Wheat flour was got from the Port Harcourt flour mills; shortening, eggs, salt and baking poweder were also purchased from the oil mill market.

Pepsin and pancreatic enzymes were also used for the study. Preparation of fluted pumpkin seed flour.

Fluted pumpkin seeds with intact seed coats were processed into flour as shown in Figure 1. The seeds were washed and boiled in water in a stainless steel kettle for 2 hours. The seeds were allowed to cool and the seed coats removed manually and oven dried (60 °C, 48 hours) in a domestic gas oven.

Dried, dehulled seeds were ground using a commercial mill and sieved through a 425 mm mesh size. The flour obtained was partially defattened by solvent extraction for 3 hours using petroleum ether (40°-60 °C) and dried in the oven for 24 hours. The flour was then stored in polythene bags at room temperature until used.

Preparation of Fluted pumpkin seed flour

**Figure 1: Flow Chart for the Production of Fluted Pumpkin Flour**

Fluted pumpkin seed
↓
Washed
↓
Boiled in tap water for 2 hours
↓
Oven dried (60 °C) 48 hours
↓
Dehulled
↓

Figure 1 (Cont.)

Milled to pass through a 425 mm mesh size
↓
Partial defattened (solven extraction)
↓
Dried (oven – 24 hours)
↓
Package (in polythene)
↓
Flour Stored (37 °C – room temperature)

### Cookies Formation and Preparation

Blends containing 0.5, 10, 15 and 20% fluted pumpkin flour replacing wheat flour were prepared by gradual mixing of fluted pumpkin flour and wheat flour in a rotary mixer (Philips, type HB 1500/A, made in Holland) as shown on Table 1. A modified sugar cookie recipe and procedure described by Mc-waters *et al.* (2003) was used for cake preparation. The basic ingredients used were 300 g of flour blend, 180 g hydrogenated vegetable shortening, 225 g of granulated sugar, 21 g of beaten whole egg, 3.75 g of salt and 1.8 g of baking powder. The dry ingredients (flour, sugar, salt and baking powder) were thoroughly mixed in a bowel by hand for 3-5 min. vegetable shortepping was added and mixed until uniform for 3-5 minutes to get a slightly firm dough.

**Table 1: Ingredients Used for Cookies Production**

| Ingredients | Cookies (g) | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Wheat flour (%) | 100 | 95 | 90 | 85 | 80 |
| Pumpkin flour (%) | 0 | 5 | 10 | 15 | 20 |
| Sugar (g) | 22.5 | 22.5 | 22.5 | 22.5 | 22.5 |
| Shortening (g) | 180 | 180 | 180 | 180 | 180 |
| Egg(g) | 21 | 21 | 21 | 21 | 21 |
| Salt (g) | 3.75 | 3.75 | 3.75 | 3.75 | 3.75 |
| Baking powder | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |

**Note:** *A = (100% wheat flour), B: (5% pumpkin, flour 95% wheat flour; C = 10% pumpkin flour, 90% wheat flour, D = 15% pumpkin flour 50% wheat (E = 20% pumpkin flour, 80% wheat flour).

*Source: Giami et al. (2005)*

The dough was manually rolled on a pastry board into sheets of uniform thickness of 0.4 cm and cut into circular shapes of 5.8 cm diameter using cookie cutters. The cut dough pieces were transferred into oil-greased pans and baked at 180 °C for 10 min, the cookies were allowed to cool at room temperature (28 $\pm$ 1 °C) for 2 hours and then, samples were milled, ashed and samples now ready for use.

## Chemical Composition

Moisture content was determined using the gravimetric method reported by Jame (1995). The AOAC (2000) furnance incineration grvimetric method was used for the content was used for the ash content determination. Crude protein content was determined by Kjeldahl method described by James (1995). The fat content of the samples were determined by the continuous solvent extraction method described by Pearson (1976) and James (1995). The Weende method described by James (1995) was used for the crude fibre determine carbohydrate content was claucated by difference (James, 1995).

## In-Vitro Protein Digestibility

In-vitro protein digestibility of the sample was determined by the method of Sauder *et al.* (1973) with slight modification in centrifugation and filtration.

Defatted flour (250 n 0.01 mg) was suspended in 15 ml of 0.1 N hydrochloric acid in a glass tube. Pepsin enzyme (1.5 $\pm$ 0.01 mg) was introduced into the suspension and shaken at 37 °C for 3 hours. The solution was neutralized and 0.5 N sodium hydroxide and treated with 4 mg of pancreatic enzyme in 7.5 ml of 0.2 ml phosphate buffer (pH 8.0) containing 0.5ml sodium azide.

The mixture was shaken gently at 37 $\pm$ 2 °C for 24 hours. The solids were separated by centrifuging at 4000 rpm for 30 minutes and washed with water (5 x 30 ml) and filtered with whatman No. 1 filter paper. The residue was dired in the over at 100 $\pm$ 2 °C and analyzed for nitrogen by the kjeldahl method. The nitrogen content of the sample was also analyzed. The invitro protein digestibility was calculated in accordance with the formula.

$$\%\ in-vitro\ \ protein\ \ digestibility = \frac{N_2\ \ of\ \ sample - N\ \ of\ \ residue \times 100}{N_2\ \ of\ \ samples}$$

## Mineral Determination

Mineral contents of the samples were determined by dry ashing extraction method (James, 1995). 2.0 g sample was ashed in a muffle furnance at 550 °C for 3 hours. The resulting ash dissolved in 10 ml of 1 MHCL acid then diluted to 100 ml in volumetric flask using distilled water. The digesta so obtained was used for the various analysis.

## Phosphorus Determination (P)

Phosphorus was determined by the Molybdoyanadata (yellow) spectrometry described by James (1995).

Phosphorus content of the test samples was calculated as show below:

$$P_{mg} \Big/ 100g = \frac{100 \times Au \times C \times Vf}{W \times As \times Va}$$

where

Au = Absorbance of test sample

C = Concentration (in mg/ml) of standard phosphorus solution

Vf = Total volume of extract

W = Weight of sample analyzed

As = Absorbance of standard phsphorus solution

Va = Volume of extract analysed

## Iron, Calcium and Zinc

The mineral were determined in the samples by the dry-ashing method described by Pratt. The amount of zinc calcium and zinc were determined using atomic absorption spectroscopy (Perkin Elmer 2380).

## Sensory Evaluation

Taste panel evaluation of cookies were carried out using eighteen consumers comprising staff of Rivers State university of Science and Technology.

Members of the panel were above eighteen years of age and are regular consumers of cookies. They were trained in the use of sensory evluaton procedures and the meaning of the terms used was described. During each assessment, the panelist were served cookies in saucers and water was given to them for proper rinsing of their mouth before the next criteria was assessed.

Panelists were askd to evaluate colour first and then, texture, flavour, taste and acceptability. A five (5) point nedonic scale with 1 = dislike, very, much, 2 = dislike slightly, 3 = indifferent, 4 = good and 5 = excellent. This evaluation was done immediately after the production of cookies.

## Statistical Analysis

The 1999 version of the Statistical Analysis System (SAS), a software package was used for the statistical analysis. Analysis of variance (ANOVA) was carried out on the data obtained from the chemical analysis, mineral contents and the invitro-protein content of the samples. Mean separation was done using fishes LSD to determine significances ($P < 0.05$).

## RESULTS

The chemical composition (% dry matter) of cookies made from wheat and fluted pumpkin flour bend is shown in Table 2. The protein levels (0%, 5%, 10%, 15% and 20% fluted pumpkin flour) of the cookies ranged from 6.125 in wheat floru products to 7.70% in the products made form the composite flour. The carbohydrate levels varied. The cookies made from wheat flour had higher carbohydrate (44.61%) than the products made from the composite flours (39.42%, 37.51%, 36.10% and 34.02%), fat levels of cookies ranged from 2.2% to 20%, while ash varied with 1.10% for the control, 2.6%, 2.4%, 1.2% and 1.3% for products made with decreasing levels of the composite flours respectively. The fiber content had a range of 1.95% and 1.56 while the moisture content of cookies varied with 5.25% for the control.

The levels of P, Fe, ca, zin are shown on Table 3.

Cookies made from the wheat had lower levels of Fe, ca, and p contain 0.14 mg, 15.8 mg and 1.22 mg respectively, while zn was higher, 1.29 mg. There, was considerable increase for cookies made from the composite flour, Fe $C_4$ 6 mg, Ca, 24.5 mg; P. 1.34 mg; while Zn was redued to 1.20 mg).

**Table 2: Chemical Composition of Confectionary Products Made from Wheat/Fluted Pumpkin Flour Blends (%)**

| Level of Fluted Pumpkin in Sample | Carbohydrate Protein Fat Moisture as Fiber | | | | | |
|---|---|---|---|---|---|---|
| 0 | $44.61^{b}$ | $6.125^{x}$ | $2.2^{z}$ | $5.25^{z}$ | $1.0^{y}$ | $1.95^{d}$ |
| 5 | $39.42^{a}$ | $6.56^{xy}$ | $2.4^{y}$ | $3.60^{yx}$ | $2.6^{x}$ | $1.80^{ad}$ |
| 10 | $37.51^{a}$ | $7.00^{y}$ | $2.4^{x}$ | $3.15^{y}$ | $2.4^{x}$ | $1.25^{af}$ |
| 15 | $36.10^{y}$ | $7.26^{yz}$ | $2.0^{y}$ | $5.80^{x}$ | $1.2^{y}$ | $1.36^{a}$ |
| 20 | $24.02^{x}$ | $7.70^{z}$ | $2.0^{y}$ | $4.40^{z}$ | $1.3^{y}$ | $1.58^{a}$ |

**Note:** Means of triplicate determinations means values with the same superscript letters within the same row do not differ ($P>0.05$).

**Table 3: Levels of Mineral in Cookies Produced from Wheat/Pumpkin Flours***

| Levels of Pumpkin Flour in Cookies | Zn (mg/100 g) | Fe (mg/100 g) | Ca (mg/100 g) | P (mg/100 g) |
|---|---|---|---|---|
| 0 | $1.29^{x}$ | $0.14^{x}$ | $15.8^{y}$ | $1.22^{x}$ |
| 15 | $1.20^{x}$ | $4.6^{x}$ | $24.5^{x}$ | $1.34^{x}$ |

**Note:** * Mean of triplicate determinations; Mean values with the same superscript letters within the same vow do not differ ($P > 0.05$).

The Table 4 lists the values obtained for protein from digestibility of the product protein digestibility of the products made from wheat flour was 18.40% and 26.10% for cookies, while the digestibility levels for products made from the composite flours was 92.63%.

**Table 4: Determination of *in Vitro* Protein Digestibility of Cookies**

| Levels of Pumpkin in Blend | % Nitrogen of Sample | % Nitrogen of Residue | % Digestibility |
|---|---|---|---|
| 0 | $1.25^{a}$ | $1.0^{a}$ | $18.40^{b}$ |
| 15 | $1.40^{c}$ | $0.084^{d}$ | $92.6^{e}$ |

**Note:** * 0 = control (100% wheat) is composite (15% fluted pumpkin flour: 85% wheat flour) mean of triplicate determination. Mean values with the same superscript letters within the same row do not differ $P > 0.05$.

**Table 5: Determination of Molar Ratios**

| Cookies % Fluted Pumpkin Blend | Phytate % | Ca | Mg | Zn | Molar Ratios |
|---|---|---|---|---|---|
| | | Mg | Mg | Mg | |
| 0 | 6.16 | 15.8 | 15.5 | 1.29 | 44 |
| 15 | 2.96 | 24.5 | 24.3 | 1.2 | 60.84 |

## DISCUSSION

The higher protein content observed in the cookies made for the composite flours, than those made from the wheat flour was likely due to the supplementation effect of the fluted pumpkin seed flour on the wheat flour. Fluted pumpkin is an oil seed with significant quantities of protein (27%) and fat (54%), Achinewhu (1986). The high fat and protein contents of fluted pumpkin seeds upgraded the levels of the nutrients in the composite flour and demonstrated the beneficial effect of blending two foods in product development. The result confirms the observation by Ihekoronye and Ngoddy (1995), that nutritional enhancement might be an advantage in the use of composite flour for baked products. The products, particularly those made from the wheat-fluted pumpkin composite flours could be valuable in the fight against Protein-Energy Malnutrition (PEM) as well as contribute to the micronutrient intake of the populance, by virtue of the and Ca contents.

The higher mineral (Ca, Fe and P) levels of the composites than the refined wheat flour products further confirms the beneficial effects of supplementation. It further supports Achinewhu (1986) who reported that the seeds are good sources of mineral such as iron and phosphorus.

The contents of antinutrints did not increase on addition, hence their inactivation is absolutely necessary to improve the nutritional quality of fluted pumpkin seed flour and effectively utilize its full potential as human food likewise more work needs to be done on the antinutrients to observed if the composite flour products will have higher levels of phitate and tannic acids. This study revealed good digestibility values for the products made from the composite flour than the control (100% wheat flour). This suggest that the nutritional quality of cookies improved at 15% supplementation with pumpkin flour. Hence, the improved in vitro digestibility of protein in the present investigation may improve the nutritional quality of wheat flour by the supplementation with fluted pumpkin seed flour in food product.

In determining the absorption of zinc, the level of calcium in the sample affected the ration at which phytate impaired zinc availability (Morris and Ellis, 1980).

It is also possible that other dietary factors such as protein content of the meal/diet, calcium concentration, etc., may affect the ratio for which an effect is observed (Fredlund, 2002). These study recommend that fluted pumpkin flour from 0 to 15% level of substitution be made for cookies production.

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