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Utilization of protein concentrates from ungerminated and germinated fluted pumpkin (*Telfairia occidentalis* Hook) seeds in cookie formulations

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Abstract: Cookies (soft type biscuits) were produced from blends of wheat flour containing graded levels (0–25%) of protein concentrates prepared from ungerminated and germinated fluted pumpkin (*Telfairia occidentalis* Hook) seeds and evaluated for nutritional, baking and sensory properties. Protein quality was investigated using weanling albino rats fed diets that were formulated to supply 10% protein using cookie samples, with casein as a control. Cookies produced from blends containing protein concentrates from germinated seeds had higher contents of crude protein and lower levels of polyphenol and phytic acid, compared with cookies supplemented with concentrates from ungerminated seeds. The use of up to 15% concentrate from ungerminated seeds in the blends produced cookies with spread ratio, hardness, colour and flavour similar to the 100% wheat flour (control) cookies. Cookies supplemented with concentrates from germinated seeds at 15–25% levels were nutritionally comparable to diets based on casein, but at the expense of acceptability.

Keywords: cookies; fluted pumpkin; protein concentrate; quality

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis* Hook) is a high protein oilseed crop adapted to the climatic and geographical conditions in the tropics. The crop has some agronomic advantages over other oilseed crops, such as drought resistance and tolerance to a wide range of soils.[1] It is the most widely cultivated leafy vegetable crop in West Africa and the crop also produces edible oil-bearing seeds. The seed has about 27% protein and 54% fat and is a good source of minerals such as iron and phosphorus.[2] The nutritional quality (amino acid composition, protein digestibility and protein efficiency ratio) of fluted pumpkin seed is similar to that of soybean.[2,3] Despite these advantages, fluted pumpkin seed has not yet become a popular part of the local diet of Nigerians. Apart from being fermented and used as a flavouring ingredient or protein supplement in some local foods,[3,4] there is limited food application of fluted pumpkin seed, compared with other major oilseeds. Producing protein concentrate from fluted pumpkin seed is one method of introducing it into the human diet. Protein concentrate from germinated fluted pumpkin seed has been reported to have lower contents of antinutrients, and improved functional and nutritional properties, compared with the flour or concentrate from ungerminated seeds.[5–7] Therefore, the development of value-added products utilizing protein concentrate from germinated fluted pumpkin seed could lead to the production of novel products, with improved nutritional value.

Cookie (soft type biscuits) are widely accepted and consumed in many developing countries. They offer a valuable vehicle for supplementation with fluted pumpkin protein concentrate for nutritional improvement. Although the use of fluted pumpkin seed in bread making has been studied,[8] cookies have been suggested as a better use of composite flour than bread due to their wider consumption and relatively long shelf life.[9,10] Protein concentrates from soybean, safflower, sunflower, fababean and great northern bean have been studied for utilization in cookie production[9,11–14] but these oilseeds and legumes are not adapted to the humid tropical Nigerian climate. The purpose of this study was to blend graded levels (0–25%) of protein concentrate prepared from ungerminated and germinated fluted pumpkin seeds with wheat flour and to examine the effects of these blends on chemical, physical, sensory and nutritional properties of cookies.

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MATERIALS AND METHODS

Materials

Commercial high quality wheat flour (plain, all purpose, golden crown brand) was obtained from Nigerian Flour Mills, Port Harcourt, Nigeria. Fluted pumpkin (*Telfairia occidentalis* Hook) fruits were obtained from the author's farm in Ogoni, Rivers State, Nigeria. The seeds were separated from the pulp and husks for use in the experiments. Weanling male albino rats (Wistar strain) were obtained from the animal colony of the University of Port Harcourt, while vitamin and mineral premixes were supplied by Rhodia Nig Ltd, Lagos Nigeria. All other reagents used were of analytical grade (BDH Chemicals, Poole, UK).

Sample preparation

Germination of the seeds was carried out as described in a previous paper.[15] The seeds were placed in sterile aluminum trays lined with wet cotton wool and germinated at room temperature ($28 \pm 1\,^\circ$C) for 72 h. The cotton wool was moistened with distilled water at regular intervals of 12 h. Germinated and ungerminated seeds with intact seed coats were boiled in distilled water in a covered stainless steel pot for 1 h (length of time required to soften the seed coats and facilitate dehulling). Cooked seeds were dehulled manually and oven-dried (60 °C, 24 h) in a hot-air fan oven (Model QUB 305010G, Gallenkamp, UK). Dried, dehulled seeds were ground using a laboratory mill (Numex Pep Mill, India) and screened through a 0.25-mm British standard sieve (Model BS 410, Endecotts, UK). Flours obtained were defatted by solvent extraction in a Soxhlet apparatus. (Tecator Inc, Colorado, USA) for 8 h using n-hexane. The defatted flours were spread on aluminum trays and dried in a hot-air fan oven (70 °C, 30 min) to expel residual hexane and stored in air-tight plastic containers at 4 °C until used.

Preparation of protein concentrates

Protein concentrates were prepared from defatted flours from ungerminated and germinated fluted pumpkin seeds, using an alkaline wet extraction process described by Deshpande and Cheryan.[16] The flour sample (50 g) was suspended in 300 ml of 0.04 M NaOH and the mixture was stirred at room temperature ($28 \pm 1\,^\circ$C) for 1 h using a mechanical shaker. The pH of the slurry was adjusted to 10.8 using 1 M NaOH and centrifuged at 5000 rev min^{-1} for 25 min to obtain a residue and a supernatant. The residue was resuspended in 200 ml of 0.04 M NaOH and the extraction procedure repeated to increase the yield of protein. The pH of the combined extracts was adjusted to 4.5 using 1 M HCl to precipitate the major proteins. The mixture was centrifuged at 5000 rev min^{-1} for 15 min to yield a precipitate (protein concentrate) which was washed twice with distilled water, adjusted to pH 7.0 using 1 M NaOH, air-dried (48 h) at room temperature ($28 \pm 1\,^\circ$C) and stored at 4 °C until used.

Preparation of cookies

Blends containing 0, 5, 10, 15, 20 and 25% fluted pumpkin protein concentrate replacing wheat flour were prepared by gradual mixing of fluted pumpkin concentrate and wheat flour in a rotary mixer. A modified sugar cookie recipe and procedure described by McWatters *et al*[17] was used for cookie preparation. The basic ingredients used were 300 g of flour blend, 180 g of hydrogenated vegetable shortening, 225 g of granulated cane 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 bowl by hand for 3–5 min. Vegetable shortening (180 g; Monita baking fat, Karya Prima, Bekasi, Indonesia) was added and mixed until uniform. Egg was then added and mixture kneaded in a Kenwood mixer (Model A 907D) for 3.5 min to get a slightly firm dough. 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 a circular scone cutter. 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 ($28 \pm 1\,^\circ$C) for 2 h and then divided into three lots. One lot was used immediately for the measurement of physical characteristics. The second lot was subjected to sensory evaluation after 20 h. The third lot was milled, screened through a 0.25-mm sieve, defatted by solvent extraction using n-hexane for 8 h and used for chemical analysis and diet formulation for rats.

Chemical analyses

Crude protein contents of cookies were determined by method 2.057 of the AOAC.[18] The factor $N \times 6.25$ was used for conversion of nitrogen to crude protein. For the determination of phytic acid, a combination of two methods was used. The extraction and precipitation of phytic acid were performed according to the method of Wheeler and Ferrel;[19] iron in the precipitate was measured using method 14.013 of the AOAC.[18] A 4:6 Fe:P molecular ratio was used to calculate the phytic acid content. Polyphenols were determined using the vanillin–H_2SO_4 assay[20] with reference to a standard graph based on phloroglucinol. The results are expressed as mg of phloroglucinol equivalents per 100 g of dry concentrate weight.

Physical properties

The physical properties of cookies which were measured as averages of five replicates for each sample were weight, diameter, height (thickness) and hardness (texture). The procedure outlined by Zoulias *et al*[21] was used for the measurement of cookie diameter. Four cookies were placed next to each other and the total diameter was measured. Then all four cookies were rotated by 90° and the new diameter was measured. The average of the two measurements divided by four was taken as the final diameter of a cookie. Spread ratio was expressed as diameter/height. Hardness was measured by a compression test using a pulley-type shear frame as described in a previous paper.[22] The mean of shear stress value of ten cookies was calculated and reported as Newtons per cookie.

Sensory analysis

A panel of twenty—six consumers comprising staff and students from the University of Science and Technology and the Port Harcourt Flour Mills, was used to evaluate sensory properties of cookies. This number of panelists is considered adequate for rough product screening and for evaluating acceptance and/or preference.[23] Criteria for selection were that panellists were 18 years of age, were regular consumers of cookies and were neither sick nor allergic to any food. Panellists were trained in the use of sensory evaluation procedures and the meaning of the descriptive terms used.

At each session, samples were served on white saucers identified with three-digit code numbers to eliminate bias. Panellists were instructed to evaluate colour first and then to taste each sample to evaluate flavour, texture and overall acceptability. A nine-point hedonic scale with 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely was used.[24] Overall acceptability of each sample was decided on the basis of the methodology described by Subba.[25] Samples that obtained 80% of the scores in the 'like (6–9 points)' hedonic region for colour, flavour and texture, were considered acceptable. Water was provided to rinse the mouth between evaluations and covered expectoration cups if they did not wish to swallow the samples.

Protein quality

Protein quality of cookies was evaluated using a rat bioassay. A basal diet was prepared based on the formulation of the AOAC[18] as described in a previous paper;[26] its composition is shown in Table 1. Eleven experimental diets (A–K) were prepared by incorporating cookie samples into the basal diet at the expense of cassava starch, such that they provided 10% crude protein in the final diets. Weanling male albino rats, 28 days old and weighing between 34 and 36 g, were grouped by randomized block design into thirteen groups on the basis of weight, such that mean initial weights did not differ by more than ± 0.5 g. Each group consisted of ten rats and was housed in individual wire-bottomed cages that allowed for easy faecal collection and the measurement of food intake. The temperature of the laboratory was $28 \pm 1\,^{\circ}$C with alternate periods of light and dark of 12 h. One group of ten rats was fed a protein-free diet which consisted entirely of the basal diet, another group was fed a casein (control) diet while the remaining eleven groups were fed the experimental diets. The rats had free access to the diets and water for 28 days, the duration of the experiment for the protein efficiency ratio (PER) study. True digestibility (TD) study was started on the 14th day of the PER study and lasted for 7 days, while the net protein ratio (NPR) determination was done on the 10th day of the PER study. Daily records on weight gain or loss, food and protein intakes and faecal output by the rats were taken and used in calculating the PER NPR and TD, using standard procedures.[27]

Table 1. Composition of basal diet

Ingredients	Amount (%)
Protein	10
Corn oil	8
Salt mixture[a]	5
Vitamin mixture[b]	1
Cellulose	1
Cassava starch	To make up 100

[a] Salt mixture (composition $100\,g^{-1}$): calcium (0.6 g), chloride (0.5 g), copper (1.0 mg), iodine (0.02 mg), iron (10 mg), magnesium (0.2 g), manganese (7.5 mg), phosphorus (0.5 g), potassium (0.5 g), sodium (0.5 g), zinc (1.8 mg).

[b] Vitamin mixture (composition $100\,g^{-1}$): vitamin A (700 iu), vitamin D (30 iu), vitamin E (6 iu), vitamin K (0.29 mg), thiamine hydrochloride (40 mg), riboflavin (1.2 mg), pyridoxine hydrochloride (0.6 mg), niacin (1.0 mg), panthothenic acid (1.2 mg), cyanocobalamin B_{12} (0.5 µg).

Statistical analysis

All analytical determinations were conducted in triplicate. Mean values were calculated and the data were subjected to analysis of variance. If a significant F test was noted, means were separated using Duncan's multiple range test (DMRT).[28] Significance was accepted at $p \leq 0.05$.

RESULTS

The crude protein, phytic acid and polyphenol contents of control (100% wheat flour) cookie were 12.3%, 5.8 mg $100\,g^{-1}$ and 0.6 mg $100\,g^{-1}$, respectively (Table 2). All the fluted pumpkin-supplemented cookies had higher contents of crude protein (15.5–25.6%), compared with the control cookie. Blending wheat flour with up to 15% protein concentrate from ungerminated fluted pumpkin seeds produced cookies with similar levels of the antinutrient with the control cookie. However, when the level of protein concentrate from ungerminated seeds in the blends was increased to 25%, there was a significant ($p < 0.05$) increase in the levels of phytic acid (8.8 mg $100\,g^{-1}$) and polyphenol (1.7 mg $100\,g^{-1}$) in the cookies, compared with level found in the control cookie. Cookies supplemented with pumpkin concentrate from germinated seeds had significantly ($p < 0.05$) higher levels of protein and lower contents of phytic acid than cookies formulated with concentrate from ungerminated seeds.

There were no significant ($p > 0.05$) differences between the values obtained for spread ratio of cookie formulations containing 5–25% fluted pumpkin protein concentrate from ungerminated seeds and the

Table 2. Chemical composition of cookies prepared with different levels of substitution of wheat flour with fluted pumpkin concentrate from ungerminated or germinated seeds[a]

Fluted pumpkin concentrate level in cookies (%)	Treatment of seeds	Crude protein (%)[b]	Phytic acid (mg 100 g^{-1})[b]	Polyphenol (mg 100 g^{-1})[b]
0 (control)	—	12.3d	5.8 b	0.6b
5	Ungerminated	15.5c	6.0b	0.6b
	Germinated	20.1b	3.6c	0.5b
10	Ungerminated	16.3c	6.2b	0.7b
	Germinated	20.4b	3.7c	0.6b
15	Ungerminated	16.8c	6.4b	0.8b
	Germinated	20.5b	3.8c	0.6b
20	Ungerminated	18.2b	8.6a	1.5a
	Germinated	25.0a	5.7b	0.7b
25	Ungerminated	18.4b	8.8a	1.7a
	Germinated	25.6a	5.9b	0.8b

[a] Mean of triplicate determinations.
[b] Means with the same letter within a column do not differ significantly ($p > 0.05$) by the Duncan's multiple range test.

Table 3. Physical characteristics of cookies prepared with different levels of substitution of wheat flour with fluted pumpkin concentrate from ungerminated or germinated seeds[a]

Fluted pumpkin concentrate level in cookie (%)	Treatment of seeds	Weight (g)[b]	Diameter (D) (cm)[b]	Height (H) (cm)[b]	Spread ratio (D/H)[b]	Hardness (N)[b]
0 (control)	—	12.5c	10.5a	1.6a	6.6a	59.4a
5	Ungerminated	12.7c	8.3b	1.3a	6.4a	57.3a
	Germinated	12.6c	8.1b	1.3a	6.2a	40.0b
10	Ungerminated	14.3b	8.2b	1.3a	6.3a	56.2a
	Germinated	14.0b	7.9b	1.3a	6.1a	38.2b
15	Ungerminated	14.8b	8.0b	1.3a	6.2a	55.4a
	Germinated	14.5b	7.4b	1.3a	5.7a	37.6b
20	Ungerminated	16.4a	7.5b	1.3a	5.8a	36.7b
	Germinated	16.1a	3.4c	1.2a	2.8b	21.8c
25	Ungerminated	16.8a	7.0b	1.2a	5.8a	34.6b
	Germinated	16.5a	3.1c	1.2a	2.6b	19.5c

[a] Mean of five determinations.
[b] Means with the same letter within a column do not differ significantly ($p > 0.05$) by the Duncan's multiple range test.

control cookie (Table 3). It was however, observed that there was a decrease in this cookie characteristic when pumpkin concentrate from germinated seed incorporated in the composite cookie increased to 20–25%. Blends containing protein concentrates from germinated seeds produced softer cookies which required less force to compress.

Cookies produced from wheat flour containing up to 15% protein concentrate from ungerminated pumpkin seeds were similar to the control (100% wheat flour) cookie with respect to colour, texture, flavour and overall acceptability (Table 4). Hardness (texture) estimated by the sensory panel was in good agreement with the measurements derived from the physical compression test. Germination of pumpkin seeds impaired sensory characteristics of cookies as shown by the low and unacceptable scores for colour, texture and flavour, compared with cookies prepared with concentrates from ungerminated seeds.

Protein quality indices of cookie diets (Table 5) showed that rats fed diet formulated with the control (100% wheat flour) cookie had low weight gain (14.1 g). In addition, the diet resulted in poor protein quality indices such as low value (1.1) for PER, low value (1.0) for NPR and a low value (62.4%) for TD. Cookies produced with protein concentrate from both ungerminated and germinated fluted pumpkin seeds had significantly ($p < 0.05$) higher PER, NPR and TD than the control (100% wheat flour) cookie.

DISCUSSION

Although soybean has been a primary source of plant protein for use as a functional agent in food systems, the crop is not adapted to varying growing conditions. Many researchers have reported the preparation and properties of protein concentrates from other plant sources.[6,9,12–14] Such efforts are aimed at effective utilization of inexpensive proteins for nutritional and functional purposes. Fluted pumpkin protein concentrate seems to offer some important advantages over many other oilseed and legume protein concentrates as

Table 4. Sensory properties[a] of cookies with different levels of substitution of wheat flour with fluted pumpkin concentrate from ungerminated or germinated seeds

Fluted pumpkin concentrate level in cookie (%)	Treatment of seeds	Colour[b]	Texture[b]	Flavour[b]	Overall acceptability[b]
0 (control)	—	8.7a	8.8a	8.6a	8.0a
5	Ungerminated	8.3a	8.2a	8.3a	7.9a
	Germinated	5. 5b	5.6b	5.2b	5.6b
10	Ungerminated	8.0a	7.9a	8.0a	7.8a
	Germinated	5.3b	5.5b	5.1b	5.5b
15	Ungerminated	7.8a	7.8a	7.8a	7.7a
	Germinated	5.2b	5.3b	5.0b	5.3b
20	Ungerminated	7.7a	5.6b	5.1b	5.3b
	Germinated	5.1b	3.0c	3.0c	3.1c
25	Ungerminated	5.3b	5.3b	3.6c	5.0b
	Germinated	3.1c	2.8c	1.4d	2.9c

[a] Mean hedonic scores,[24] where 9 = like very much, 1 = dislike very much.
[b] Means with the same letter within a column do not differ significantly ($p > 0.05$) by the Duncan's multiple range test.

Table 5. Protein quality attributes[a] of diets containing cookies prepared with different levels of substitution of wheat flour with fluted pumpkin concentrate from ungerminated or germinated seeds fed to rats

Diet[b]	Weight gain (g)	Protein intake (g)	Protein efficiency ratio	Net protein ratio	True digestibility (%)
A	14.1d	13.1b	1.1c	1.0d	62.4c
B	17.0c	13.1b	1.3b	1.4c	73.1b
C	19.3c	14.9b	1.3b	1.7c	74.2b
D	17.6c	14.1b	1.2b	2.1 b	75.2b
E	21.0c	14.2b	1.5b	2.6b	77.5b
F	20.0c	14.5b	1.4b	2.3b	76.2b
G	33.8b	14.6b	2.3a	4.1a	89.9a
H	21.8c	15.2b	1.4b	2.4b	78.8b
I	36.9b	15.7b	2.4a	4.2a	90.6a
J	25.4c	16.7b	1.5b	2.4b	79.5b
K	40.7b	17.1b	2. 4a	4.3a	91.2a
L	54.9a	22.1a	2.5a	4.5a	94.7a

[a] Mean of ten rats per group. Means with the same letter within a column do not differ significantly ($p > 0.05$) by the Duncan's multiple range test.
[b] Prepared from cookies containing 0% (A), 5% ungerminated (B) or germinated (C), 10% ungerminated (D) or germinated (E), 15% ungerminated (F) or germinated (G), 20% ungerminated (H) or germinated (I), 25% ungerminated (J) or germinated (K) fluted pumpkin concentrates; L = casein (control).

a food ingredient. A relatively lower level ($0.98\,\mathrm{mg\,g^{-1}}$) of phytic acid has been reported[6] for pumpkin protein concentrate than the levels ($11.4–15.2\,\mathrm{mg\,g^{-1}}$) reported[16] for protein concentrates from various cultivars of dry bean. A water absorption capacity of $5.6\,\mathrm{g\,g^{-1}}$ has been reported for pumpkin protein concentrate,[6] and this value is higher than the values ($3.5–3.9\,\mathrm{g\,g^{-1}}$) reported for soybean, sunflower and winged bean protein concentrates.[29] Water absorption characteristics have been reported to represent the ability of a product to associate with water under conditions where water is limiting, such as doughs and pastes.[30] Fluted pumpkin protein concentrate is, therefore, expected to be useful as a functional agent in bakery products such as cookies. The protein content (15.5–25.6%) of the cookie formulations prepared in this study were higher than the protein levels (6–12%) reported for conventional wheat flour cookies[31,32] and other oilseed-supplemented wheat flour-based snack foods in Nigeria.[33] All the fluted pumpkin concentrate-supplemented cookies were considered to be nutritious since the consumption of about 100 g of each product formulation would provide more than half of the daily requirement for protein ($25–30\,\mathrm{g\,day^{-1}}$) recommended by FAO/WHO[34] for children aged between 5 and 19 years. Recent recommendations of protein contents in cookies used in food aid programmes are 15.1–20.0%.[35] The protein values of the pumpkin concentrate-supplemented cookies in the present study were in the similar range. The levels ($3.6–8.8\,\mathrm{mg}\ 100\,\mathrm{g^{-1}}$) of phytic acid in the pumpkin concentrate-supplemented cookies were within the range ($0–20\,\mathrm{mg}\ 100\,\mathrm{g^{-1}}$) reported for bakery products, such as wheat white bread.[36]

Cookies prepared with concentrates from germinated seeds exceeding 15% of fluted pumpkin protein concentrate had reduced spread ratios. Other workers also observed reduced spread ratios in cookies prepared with protein concentrates from great northern bean[14] and soybean.[37] Increasing the amount of shortening and addition of 0.5% of a mono- and diglyceride emulsifier has been shown to improve the spread factor of cookies.[37] It has been suggested[11] that rapid partitioning of free water to hydrophilic sites during mixing increased dough viscosity, thereby limiting cookie spread. Studies on the utilization of protein concentrates from various oilseeds and legumes in cookie formulations have been made by various researchers.[11–14] These researchers observed that the physical and other characteristics of cookies varied widely and attributed this to differences in the cookie formulations (recipes) and extraction methods used for each protein concentrate.

Acceptable cookies were produced from wheat flour–pumpkin concentrate blends containing 5–15% protein concentrate from ungerminated seeds. The low acceptability of cookies from blends containing protein concentrate from germinated seeds or more than 15% concentrates from ungerminated seeds were attributed by the panelists to a beany flavour and darkening. Colour darkening of cookies is attributed to sugar caramelization and mallard reactions between sugars and amino acids.[38] Flavour was a major factor in determining acceptability of cookies supplemented with pumpkin concentrates. This implies that there is a need to seek additional processing methods or to use additives that will improve the performance of these protein concentrates. Heat treatment, such as steaming for 30 min at 100 °C, was effective in improving the flavour of cowpea flour used in cookie preparation.[39] Studies have shown that replacement of shortening by polydextrose (Litesse) or a blend of microparticulate whey proteins and emulsifiers (Simplesse) improved the sensory characteristics and physiochemical properties of cookies.[21] The adverse effect of germination of some oilseed/legume flours and protein isolates on the sensory properties and baking characteristics of bakery products, such as bread, has been reported by other researchers.[40,41]

Blending wheat flour with 5–25% protein concentrates from ungerminated and germinated fluted pumpkin seeds resulted in cookies with significantly ($p < 0.05$) higher values for protein efficiency ratio, net protein ratio and true digestibility compared with the control (100% wheat flour) cookie. Cookies supplemented with pumpkin concentrates from germinated seeds at 15–25% levels were nutritionally comparable with diets based on casein.

CONCLUSION

This study has shown that wheat flour supplemented at 5–15% levels with protein concentrates from ungerminated fluted pumpkin seeds produced acceptable cookies. Cookies with improved nutritional quality were obtained when wheat flour was supplemented at 20–25% levels with protein concentrates from germinated seeds. This indicates that the under-utilized fluted pumpkin seeds available in Nigeria could be processed and used with advantage to produce value-added products to combat malnutrition. However, to incorporate the advantages of the germinated fluted pumpkin seed into an acceptable cookie, further studies, such as the use of additives, will be necessary.

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