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Dough Rheology and Quality of Wheat Bread Supplemented with Fluted Pumpkin Seed Protein Concentrate

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ABSTRACT

The effect of blending wheat flour (WF) with protein concentrate (PC) from fluted pumpkin *(Telfairia occidentalis* Hook) seeds at levels of 0-25% on rheological properties of dough and bread making properties were investigated. Dough rheology was studied using a Chopin alveograph and included resistance to extension (R), extensibility (E) and mechanical work for dough deformation. (W). Bread loaves were prepared using the straight –dough procedure and evaluated for baking quality, chemical composition and sensory properties. Protein quality was estimated using weanling male albino rats fed composite bread diets (from each blend) which were formulated to supply 10% protein, with casein diet as a control. There were no significant ($p>0.05$) differences between alveograph values obtained for E and W in blends containing 0 (100% WF) and 5-15% PC. Up to 15% substitution of WF with PC produced acceptable bread loaves with loaf volume, crust colour, crumb colour, crumb texture and overall acceptability similar to the control (100WF) bread. When WF was replaced with 15% PC, there was an increase of 115.4% in protein, 71.2% in calcium, 169.2% in iron and 140.8% in potassium contents of composite breads. The values obtained for protein efficiency ratio, net protein ratio and true digestibility of bread diets containing 15% PC and casein were similar, suggesting an improvement in the nutritional quality of PC-substituted composite breads.

KEYWORDS: Bread quality, Dough rheology, Fluted pumpkin, Protein concentrate.

INTRODUCTION

In many developing countries, protein-calorie malnutrition is still common due to the high cost and inadequate supply of protein from animal sources. This has created a need for the study of the food composition and potential utilization of protein from locally available food crops, especially from underutilized high protein oilseeds and legumes (Giami & Wachuku, 1997; Enujiugha & Ayodele – Oni, 2003). Fluted pumpkin (*Telfairia occidentalis* Hook) seed is one of such relatively neglected, but potentially valuable as a high protein oilseed for use as flavouring agent or food protein supplement in many tropical countries (Longe, et al, 1983; Barber, et al, 1989). It has been reported that fluted pumpkin seed protein concentrates possessed higher crude protein contents, had improved nutritional and functional properties and were relatively free of antinutrients, compared with the original flours. (Giami & Bekebain, 1992; Giami & Isichei, 1999, Giami, 2003). Therefore, the development of value-added products using fluted pumpkin protein concentrate could lead to the production of novel products with improved nutritional value.

Wheat bread is widely accepted and consumed in many developing countries, and, therefore offer a valuable vehicle for supplementation with fluted pumpkin protein concentrate for nutritional improvement. Protein concentrates from various oilseeds and legumes have been studied for utilization in bread (Yue, et al., 1991; El-soukkary, 2001). Although the use of fluted pumpkin seed flour in bread making has been studied (Giami, 2001), information on the use of fluted pumpkin concentrate is lacking. The purpose of this study was to investigate the rheological properties of wheat flour-fluted pumpkin protein concentrate blends, baking, nutritional and sensory properties of baked breads.

MATERIALS AND METHODS

Materials

Commercial wheat flour (plain, golden crown, brand) was obtained from Nigerian Flour Mills, ltd., 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 and used in the experiments. Weanling male albino rats (Wistar strain) were supplied by the animal colony of the University of Port Harcourt; vitamin and mineral premixes were from Rhodia Nig. Ltd., Lagos, Nigeria. All other reagents used (BDH Chemicals Ltd., Poole, England) were of analytical grade.

Sample preparation

Fluted pumpkin seeds, with intact seed coats, were boiled in a covered stainless steel pot for 1h (length of time required to soften the seed coats and facilitate dehulling). The cooked seeds were dehulled manually and oven-dried (60^{0}C, 24h) in a hot air fan oven (Model QUB 305010G, Gallenkamp, UK). Dried, dehulled seeds were ground using a laboratory mill (Numex Pep Mill, India) screened through a 0.25mm 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^{0}C, 30

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Table 1: Composition of diets for rat bioassay

	Diet[1]					
Ingredients (g/100g)	A	B	C	D	E	F
Bread sample	72.8	60.3	51.5	45.0	-	-
Corn oil	8.0	8.0	8.0	8.0	8.0	8.0
Salt mixture[2]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mixture[3]	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose	1.0	1.0	1.0	1.0	1.0	1.0
Cassava starch	12.2	24.7	33.5	40.0	72.5	85.0
Casein	-	-	-	-	12.5	-

[1]prepared from wheat bread supplemented with 0% (A), 5% (B), 10% (C) and 15% (D)pumpkin concentrates; E = casein; F = non-protein diet.

[2]Salt mixture (composition/100g): Calcium (0.6g); chloride (0.5g); copper (1.0mg); iodine (0.02mg); iron (10.0mg); magnesium (0.2g); manganese (7.5mg); phosphorus (0.5g); potassium (0.5g); sodium (0.5g); zinc (1.8mg).

[3]Vitmin mixture (composition/100g): Vitamin A (700 i.u); vitamin D (30 i.u);
Vitamin E (6 i.u); Vitamin K (0.29mg); thiamine hydrochloride (40mg); riboflavin (0.5mg); pyridoxine hydrochloride (0.6mg); niacin (1.0mg); pantothenic acid (1.2mg); cyanocobalamin, B_{12} (0.5 μg)

min) to expel residual hexane and used for preparation of protein concentrate.

Preparation of protein concentrate

Protein concentrate was prepared from defatted flours using an alkaline wet extraction process described in a previous paper (Giami & Isichei, 1999). Flour sample (50g) was suspended in 300ml of 0.04M NaOH and the mixture was stirred at room temperature (28±1^{0}C) for 1 h using a mechanical shaker. The pH of the slurry was adjusted to 10.8 using IM NaOH and centrifuged at 5000 rpm for 25min to obtain a residue and a supernatant. The residue was resuspended in 200ml 0.04M NaOH and the extraction procedure repeated to increase the yield of protein. The pH of the combined extracts was adjusted to 4.5 using IM HCl to precipitate the major proteins. The mixture was centrifuged at 5000 rpm for 15 min to yield a precipitate (protein concentrate) which was washed twice with distilled water, adjusted to pH 7.0 using 1M NaOH, air-dried (48h) at room temperature (28±1^{0}C) and stored at 4^{0}C until used.

Preparation of wheat flour (WF) – protein concentrate (PC) blends

Blends containing 5, 10, 15, 20 and 25% of PC replacing WF were prepared by gradual mixing of PC into WF in a Chopin MR 2L rotary mixer.

Dough rheology

Rheological properties of doughs from the blends were recorded on the Chopin alveograph (Model MA 82, Villeneuve, France) using standard recommended procedures (Launay, Bure, & Praden, 1977). Flour blend (250±0.5g) was kneaded with 130.6 ml of 2.5% (w/v) sodium chloride solution in the alveograph mixer. A mixing time of 8 min at 29^{0}C and a 20-min rest period were the conditions utilized. The dough was inflated to its breaking point at an air flow rate of 50cm^3 sec^{-1} and the extent to which the dough was stretched (deformation measurements) was recorded on a graph paper. From the alveograms obtained, rheological properties of dough were determined. They included resistance (R) of dough to deformation (height, mm, of the curve), extensibility (E) of dough (length, mm, of the curve), mechanical work W (work input, 10^{-4} joules/g) and the height/length ratio of the curve, R/E.

Bread-baking

Baking was carried out in the cereal laboratory at the Nigerian Flour Mills Ltd., Port Harcourt. Nigeria. The WF and WF – PC blends were baked using the straight-dough method (Chauhan, Zillman, & Eskin, 1992), with little modification. The baking formula was 500g of flour blend, 9g of compressed baker's yeast, 5g of NaCl, 13g of sugar, 10g of vegetable shortening and 280ml (approximately) of water. All the ingredients were mixed in a Kenwood mixer (Model A 907 D) for approximately 3.5 min at low speed. The doughs were fermented for 90 min at 30^{0}C, 85% relative humidity and baked at 250^{0}C for 30 min. Baked breads were divided into three lots. One lot was used for the measurement of bread characteristics; the second lot was used for sensory evaluation while the third lot was milled, screened through a 0.25mm sieve, defatted by solvent extraction using n-hexane for 8h and used for chemical analysis and diet formulation for rat bioassay.

Chemical analyses

Moisture, crude protein and ether extract contents of breads were determined by the AOAC (1984) standard methods 14.004, 2.057 and 7.062, respectively. The factor N x 6.25 was used for conversion of nitrogen to crude protein. Mineral analysis was done by dry ashing (method 14.013) procedure of the AOAC (2000). Calcium, iron, potassium and sodium were determined using an atomic absorption spectrophotometer, AAS (Model 372, Perkin – Elmer Ltd., Beaconsfield, UK). Phosphorus was determined by the molybdovanadate method, using procedure 7.125 of the AOAC (2000). Phytic acid was determined using a combination of two methods,. The extraction and precipitation of phytic acid were performed according to the method of Wheeler, & Ferrel (1971); iron in the precipitate was measured using method 14.013 of the AOAC (2000). A 4:6 Fe/P molecular ratio was used to calculate the phytic acid content.

Baking quality of bread

Bread baking quality was evaluated by measuring loaf weight, loaf volume and specific volume, 30 min after removal from the oven. Loaf volume was measured by the seed displacement method using pearled barley in place of rapeseed, while specific volume was obtained by dividing the loaf volume by its corresponding loaf weight.

Sensory evaluation

A twenty–six member consumer panel comprising staff and students from the University of Science and Technology and the Port Harcourt Flour Mills was used to evaluate sensory properties of bread, 20h after baking. Panellists attended four sessions and at each session, samples (two sliced pieces of bread of uniform size and thickness) were served on white saucers identified with 3-digit code numbers to eliminate bias. Water was provided to rinse the mouth between evaluations and covered expectoration cups if they did not wish to swallow the samples. Each sample was rated, with reference to the control (100% wheat flour) bread, for crust colour, crumb colour, crumb texture, flavour and overall acceptability on a hedonic scale of 1 to 9, where I = dislike extremely and 9 = like extremely (Larmond, 1977).

Evaluation of protein quality

Protein quality of diets containing wheat bread supplemented with 0-15% fluted pumpkin protein concentrates were evaluated by a rat bioassay and compared with casein. A 10% protein diet was formulated using defatted flour from each bread sample and casein based on the formulation of the AOAC (1984) as described in a previous paper (Giami, 2003). The composition of the experimental diets supplemented with 0% (sample A), 5% (sample B), 10% (Sample C), 15% (Sample D) pumpkin concentrates, casein (control) diet (sample E) and a non-protein (basal) diet (sample F) is shown in Table 1. Weanling male albino rats of the Wistar strain, 28 days old, and weighing between 34 and 37g were randomly divided into six groups of ten replicate rats on the basis of weight, such that mean initial weights in any group did not differ by more than ±1.0g. The rats were housed in individual wire-bottom galvanized steel cages that allowed for easy faecal collection and the measurement of food intake. After an acclimatization period of three days during which the rats were fed standard stock diet, one group was fed a non-protein diet (sample F), another group was fed a casein (control) diet (sample E) while the remaining four groups were fed one of the experimental bread diets (A-D). The temperature of the laboratory was at 28±1°C throughout the period of the experiment with alternate periods of light and dark of 12h. 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 recommended equations (Pellet & Young, 1980).

Table 2: Rheological properties of doughs prepared from wheat flour –fluted pumpkin protein concentrate blends

	Chopin alveograph values[1]			
Pumpkin concentrate level in blend (%)	Resistance to deformation (R) (mm)	Extensibility (E) (mm)	Mechanical work (W) ($x10^{-4}$ joules/g)	R/E ratio
0 (Control)	$120.4^a±2.0$	$66.5^a±1.2$	$315.0^a±2.1$	$1.81^a±0.02$
5	$117.6^a±1.5$	$65.3^a±1.0$	$302.4^a±1.8$	$1.80^a±0.01$
10	$116.0^a±1.5$	$65.3^a±1.0$	$226.9^a±1.2$	$1.78^a±0.03$
15	$95.7^b±0.5$	$63.8^a±0.8$	$220.7^a±0.8$	$1.50^a±0.01$
20	$52.3^c±0.6$	$53.4^b±0.5$	$116.2^b±1.0$	$0.98^b±0.04$
25	$30.4^d±0.3$	$31.6^c±0.8$	$98.4^c±0.6$	$0.96^b±0.02$

[1]Mean± standard deviation of triplicate determinations; means with the same superscript within the same column do not differ (p >0.05).

Table 3: Chemical composition of wheat flour – fluted pumpkin concentrate composite breads[1]

Component	Pumpkin concentrate level in bread (%)					
	0	5	10	15	20	25
Moisture (%)	$12.7^{a}\pm0.6$	$12.6^{a}\pm0.4$	$12.4^{a}\pm0.3$	$12.3^{a}\pm0.1$	$12.3^{a}\pm0.1$	$12.1^{a}\pm0.2$
Crude protein (%)	$10.4^{c}\pm0.3$	$17.2^{b}\pm0.5$	$19.0^{b}\pm0.4$	$22.4^{a}\pm0.3$	$22.7^{a}\pm0.4$	$23^{a}.0\pm0.6$
Ether extract(%)	$1.6^{a}\pm0.2$	$1.6^{a}\pm0.2$	$1.6^{a}\pm0.2$	$1.7^{a}\pm0.1$	$1.7^{a}\pm0.1$	$1.7^{a}\pm0.1$
Minerals (mg/100g):						
Calcium	$49.7^{c}\pm0.7$	$69.3^{b}\pm0.8$	$73.5^{b}\pm0.6$	$85.1^{a}\pm0.5$	$86.2^{a}\pm0.6$	$86.8^{a}\pm0.7$
Sodium	$332.8^{b}\pm1.2$	$347.1^{b}\pm1.2$	$416.3^{a}\pm1.0$	$418.2^{a}\pm0.8$	$419.4^{a}\pm0.9$	$421.3^{a}\pm1.1$
Potassium	$99.8^{c}\pm0.5$	$171.6^{b}\pm0.7$	$179.5^{b}\pm0.6$	$240.3^{a}\pm1.0$	$248.1^{a}+1.1$	$249.8^{a}\pm0.8$
Iron	$1.3^{b}\pm0.1$	$1.5^{b}\pm0.1$	$1.8^{b}\pm0.6$	$3.5^{a}\pm0.2$	$3.7^{a}\pm0.3$	$3.7^{a}\pm0.3$
Phosphorus	$112.3^{c}\pm0.7$	$186.9^{b}\pm0.9$	$190.3^{b}\pm0.6$	$231.4^{a}\pm1.0$	$236.0^{a}\pm0.9$	$238.4^{a}\pm1.2$
Antinutrient (mg/100g)						
Phytic acid	$5.6^{a}\pm0.2$	$5.6^{a}\pm0.2$	$5.7^{a}\pm0.1$	$5.8^{a}\pm0.1$	$5.9^{a}\pm0.2$	$6.0^{a}\pm0.1$

[1]Mean ±standard deviation of triplicate determinations; means with the same superscript within the same row do not differ ($p>0.05$).

Statistical analyses

All experiments were conducted in triplicate. The mean±standard deviation of three values were calculated. Data were subjected to analysis of variance. If a significant F test was noted, means were separated using Duncan's Multiple Range Test (Wahua, 1999). Significance was accepted at 0.05 level of probability.

RESULTS AND DISCUSSION

Dough rheology

Resistance to extension (R), extensibility (E) and mechanical work of deformation (W) of the doughs decreased with increases in the level of pumpkin concentrate in the blends (Table 2). There were no significant ($p>0.05$) differences between the alveograph values for E and W in blends containing 0 (100% wheat flour) and up to 15% pumpkin concentrate. The W, a measure of the energy for dough deformation, decreased from 315.0×10^{-4} joules/g in the control blend to 220×10^{-4} joules/g at the 15% level of blending with pumpkin concentrate, representing a 30% decrease. A decrease in W-value amounting to 38% was reported by Misra, Usha, & Singh (1991) for wheat - soyflour blend at 10% replacement level and attributed to a weakening and destabilization of wheat flour gluten following the incorporation of soybean protein. Studies on bread making properties of pumpkin (*Cucurbita moschata*) seeds by El-Soukkary (2001) showed that dough weakening in wheat flour – pumpkin protein concentrate blends could have been due to (a) the presence of sulphydryl groups in pumpkin seed products which led to dough softening, (b) the decrease in wheat gluten because of dilution effect and (c) the competition between proteins of pumpkin products and wheat flour for water.

Reduced dough extensibility observed in this study in blends containing high amounts (more than 15%) of fluted pumpkin protein concentrate were also observed in wheat flour - sunflower protein concentrate blends (Yue, et al , 1991). The ratio of the resistance to extension of a dough (R) and extensibility (E), the R/E ratio, has been shown to give sufficient information on the dough quality of a flour or flour blend. For dough of well balanced gluten characteristics which is good in bread making, R/E ratios of 1.2 and above is recommended (Kent-Jones, & Amos, 1967). Blends containing 5-15% fluted pumpkin protein concentrates used in this study had R/E ratios greater than the recommended value of 1.2, indicating that the use of these blends in bread making is feasible.

Chemical composition of breads

The protein contents (17.2-23.0%) of pumpkin concentrate – supplemented breads (Table 3) were higher than the protein levels (10-13%) in conventional white breads (Egan et al 1981). This high level of protein is nutritionally significant since bread consumption is still high among children in developing countries. All the fluted pumpkin protein concentrate – supplemented breads (containing 5-25% pumpkin concentrate) were considered to be nutritious since the consumption of about 100g of each produced formulated is expected to provide more than half of the recommended daily requirement for protein (25-30g/day) as recommended by FAO/WHO (1973) for children aged between 5 and 19 years.

Supplementation of breads with fluted pumpkin

Table 4. Baking quality and sensory properties of wheat flour-fluted pumpkin concentrate composite breads

Pumpkin concentrate level in bread (%)	Baking quality[1] Loaf volume (cm³)	Loaf weight (g)	Specific Volume (cm³/g)	Mean hedonic scores[2] Crust colour	Crumb colour	Crumb texture	Flavour	Overall acceptability
0 (Control)	1000.5[a]	190.5[b]	5.3[a]	8.4[a]	8.2[a]	8.4[a]	8.2[a]	8.3[a]
5	998.0[a]	195.7[b]	5.1[a]	8.1[a]	8.0[a]	8.2[a]	7.9[a]	8.1[a]
10	992.6[a]	199.3[b]	5.0[a]	8.0[a]	7.8[a]	8.0[a]	7.7[a]	7.9[a]
15	988.4[a]	201.7[b]	4.9[a]	7.9[a]	7.7[a]	7.[a]	5.4[b]	7.7[a]
20	6.50.4[b]	236.5[a]	2.8[b]	5.2[b]	5.1[b]	5.2[b]	3.5[c]	5.3[b]
25	625.0[b]	245.1[a]	2.5[b]	3.4[c]	3.5[c]	5.1[b]	1.4[d]	5.0[b]

[1]Mean of triplicate determinations; [2] Where 9 = like very much, 5 = neither like nor dislike, I = dislike very much; means with the same superscript within the same column do not differ ($p > 0.05$).

Table 5. Protein quality of experimental diets containing wheat flour-fluted pumpkin protein concentrate composite breads fed to rats

Protein quality indices [1]	Pumpkin concentrate level in bread 0	5	10	15	Casein
Weight gain (g)	10.02[d]±0.12	19.65[c]±0.77	35.04[b]±0.54	49.72[a]±0.80	54.86[a]±1.00
Protein intake (g)	13.55[c]±0.10	16.36[b]±0.82	19.37[a]±0.44	21.16[a]±0.43	22.12[a]±0.04
Protein efficiency ratio	0.74[c]±0.04	1.20[b]±0.06	1.81[b]±0.04	2.35[a]±0.02	2.48[a]±0.08
Net protein ratio	0.79[d]±0.01	1.92[c]±0.07	2.98[b]±0.11	4.29[a]±0.06	4.45[a]±0.09
True digestibility (%)	60.80[d]±1.00	77.40[c]±92	89.56[b]±1.82	93.74[a]±1.22	94.64[a]±1.52

[1]Mean ±standard deviation of ten rats per group; means with the same superscript within the same row do not differ ($p>0.05$).

protein concentrate at 15%-25% level significantly ($p<0.05$) increased the levels of minerals, such as, calcium, sodium, potassium, iron and phosphorus. Increases amounting to 71.2% in calcium, 25.7% in sodium, 140.8% in potassium and 169.2% in iron contents when the level of pumpkin concentrate in bread was increased from 0% to 15%. Similar, increases in mineral contents of breads were reported by other workers when wheat flour was supplemented with pumpkin protein concentrate (El-Soukkary, 2001). The levels of phytic acid (5.6-6.0mg/100g) found in the pumpkin concentrate – supplemented breads were within the range (0-20mg/100g) reported for wheat white bread (Lopez, et al 2002). The presence of phytic acid has been identified as one of the major drawbacks limiting the nutritional quality of oilseeds and legumes by lowering the bioavailability of minerals, such as, calcium, iron and potassium (Lopez et al, 2002). Therefore, the bioavalability of these minerals in fluted pumpkin protein concentrate – substituted breads may need to be further investigated.

Baking quality and sensory characteristics

There were no significant ($p>0.05$) differences between bread loaves made from wheat flour blends containing 5-15% fluted pumpkin protein concentrate and the control bread (100% wheat flour bread) with respect to loaf volume, loaf weight, crust colour, crumb, texture and overall acceptability (Table 4). However, there were significant decreases in these bread characteristics when pumpkin concentrate incorporated in the blends increased beyond 15%. Reduction in bread volume as a result of blending wheat flour with more than 10% protein concentrate has been reported for sunflower (Yue et al, 1991). Studies by El-Soukkary (2001) showed no significant reduction in loaf volumes of breads containing up to 17% pumpkin concentrate.

The low overall acceptability of the breads baked from blends containing more than 15% fluted pumpkin concentrate was attributed by the panellists to a beany flavour and darkening. Flavour was a prominent factor in determining acceptability. At the 5-10% level of incorporation of fluted pumpkin protein concentrates in wheat flour blends, the flavour scores of the composite breads were acceptable and comparable to the 100% wheat flour blend. When fluted pumpkin concentrate was increased to higher levels, however, flavour scores dropped to unacceptable levels.

Protein quality of breads

Rats fed diet formulated with 100% wheat flour bread had a low weight gain (10.02g) and the diet resulted in poor protein quality indices, such as low values for protein efficiency ratio (0.74) (PER) net protein ratio (0.79) (NPR) and true digestibility (60.8%) (TD). Fluted pumpkin concentrate – supplemented breads at 5 to 10% levels had significantly ($p<0.05$) higher PER, NPR and TD than the 100% wheat flour bread, indicating that the nutritional quality of bread improved following a 5-10% supplementation with pumpkin concentrate. However, fluted pumpkin concentrate – supplemented breads at 15% level were nutritionally comparable to diets based on casein.

CONCLUSIONS

This study has shown that wheat flour supplemented at 5-15% levels with protein concentrate from fluted pumpkin seeds produced acceptable bread with increased protein, calcium, potassium and phosphorus contents. Also, diets formulated with composite breads supplemented at the 15% level with fluted pumpkin concentrate were nutritionally comparable to a diet based on casein.

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