Journal of Food Research; Vol. 4, No. 2; 2015
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Physico Chemical Properties and *in-vitro* Protein Digestibility of Non-Wheat Cookies Prepared From Plantain Flour and Bambara Groundnut Protein Concentrate

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Received: November 21, 2014 Accepted: December 22, 2014 Online Published: January 8, 2015

doi:10.5539/jfr.v4n2p78 URL: http://dx.doi.org/10.5539/jfr.v4n2p78

Abstract

Plantain flour was prepared from matured-unripe fruits of Agbagba cultivar, protein concentrate was prepared from bambara groundnut seeds using the alkaline extraction method, plantain cookies were produced using different levels of plantain flour substituted with bambara groundnut protein concentrate ranging from 0-25% and using 100% wheat flour as control. Physical characteristics, proximate composition, sensory properties and in-vitro protein digestibility of the cookie samples were determined. Cookies prepared from 15% bambara groundnut protein concentrate and 85% plantain flour compared favourably in physical characteristics (weight, height, diameter and hardness) with the control (100% wheat flour). Addition of bambara groundnut protein concentrate significantly improved the crude protein content (17.8%), ash content (2.8%), crude fibre (9.2%) and energy (434.0 kcal/100 g) of the cookies compared to values obtained from 100% wheat flour. Sensory evaluation showed that cookies with 15% bambara groundnut protein concentrate and 85% plantain flour was preferred in terms of colour, flavor and general acceptability with mean scores of 8.1, 8.3 and 7.8, respectively and showed no significant difference ($P \leq 0.05$) with the control with mean scores of 8.6, 8.5 and 8.0, respectively. However, in-vitro protein digestibility of the cookies increased from 2.74% in cookies with 100% plantain flour to 62.81% in samples with 25% bambara groundnut protein concentrate and 75% plantain flour.

Keywords: protein concentrate, enriched cookies, sensory properties, physical properties, protein digestibility

1. Introduction

Cookies are consumed all over the world as snack food by children and adult alike. Cookies are a form of confectionery product dried to a low moisture content (Okaka, 2009), softer when compared to biscuits. Cookies had been suggested as a better form of composite food than bread because of its ready to eat nature, wide consumption by different categories of people and relatively long shelf-life (Tsen et al., 1973). These characteristics make protein-rich cookies attractive in countries where protein energy malnutrition is prevalent (Chinma & Gernah, 2007), and also in areas needing child feeding programmes, low income and disaster relief operations (Young et al., 1985). Cookies with high sensory ratings have been produced from blends of wheat/cowpea flours (Okaka & Isieh 1990), wheat/soybean (McWatters et al., 2003), wheat and full fat soya (Ndife et al., 2014). Several studies have reported the use of wheat-based composite flour in cookie productions (Kamaljit et al., 2010; Onoja et al., 2010; Ajankau et al., 2011). All these efforts were aimed at improving the nutritional content of the cookies and also to enhance crop utilization. Recently, attempts had been made to produce cookies from non-wheat based composite flours with high nutritional and sensory properties from unripe plantain and defatted sesame flour blends (Chinma et al., 2012), cassava groundnut – corn starch blends (Agriga & Iwe, 2009) and pigeon pea, cocoyam and sorghum flour blends (Okpala & Okoli, 2011). A current trend in nutrition is the consumption of functional foods – (Foods that not only supply basic nutrients but also help to prevent disease) advocated by world nutrition bodies due to different health problem related with wheat consumption such as celiac disease, diabetes and coronary heat diseases (WHO/FAO, 2003). This situation has created the need for the consumption of low-carbohydrate diets, slowly digested starchy foods as well as an increased intake of functional foods (Hurs & Martin, 2005). Therefore, this study is aimed at producing plantain

cookies enriched with bambara groundnut protein concentrate, determine its physico-chemical properties and in-vitro protein digestibility in order to improve the nutritional quality of the cookies and increase the utilization of bambara groundnut flour.

2. Materials and Methods

A local cultivar (Agbagba) of Plantain (*Musa paradisiaca*) was harvested from the International Institute for Tropical Agriculture (IITA), high Rainfall Station, Onne, near Port Harcourt, Nigeria. Bambara groundnut (*Vigna Subterrenea (L) verdc*) seeds – (The cream coloured variety) were purchased from markets in Enugu, Nigeria. Pepsin (0.4 unit/mg) cat no p6887) and pancreatin (Cat No P 1750) enzymes were purchase from Sigma-Aldrich Chemical Ltd, U.S.A. All other chemicals used were of analytical grade.

2.1 Preparation of Plantain and Bambara Groundnut Flours

The methods of Adeniji et al. (2007) and Barimalaa et al. (1994) were used in the production of plantain flour and bambara groundnut flour, respectively.

2.2 Plantain Flour

Plantain fruits (agbagba cultivar) obtained from hand number 2, from the proximal end of the bunch, as recommended by Baiyeri and Ortiz (1996) were peeled manually with the aid of stainless steel kitchen knives and the pulp was cut into uniform slices with thickness of about 1.5mm, socked in 1.25% sodium metabisulphide solution for 5 min, drained and then dried in air circulating oven (Gallemtanp S/No 90/20/190, U.K) at 65 ºC for 20 h. The dried samples were milled to pass a 0.25 mm sieve.

2.3 Bambara Groundnut Flour

Bambara groundnut beans were sorted and the bean soaked for 24 h in tap water and dehulled manually. The seeds were further boiled for 10 min (1:4 bean to water ratio); in a stainless steel pot, drained and dried at 50 ºC in air circulating oven for 19 h. The dried samples were milled (Foss, Cyclotec 1093, Sweden) and sieved into flour using 0.25 mm sieves.

2.4 Preparation of Bambara Groundnut Protein Concentrates

The protein concentrates from bambara groundnut flour were prepared using the alkaline wet extraction process described by Giami and Isichei (1999), for fluted pumpkin seed as shown in Figure 1. About 50 gm flour sample was weighed and suspended in 300ml of 0.04M NaOH and the mixture was stirred at room temperature (28 ± 1 ºC) for 1h, using a mechanical shaker. The pH of the slurry was adjusted to 10.8 using 1.0M NaOH and centrifuged at 3500 × g for 25 min to obtain a residue and a supernatant. The residue was re-suspended in alkaline solution and the extraction procedure repeated to increase the yield of protein. The pH of the combined extract was adjusted to 4.5 using 1M HCl to precipitate more proteins. The mixture was centrifuged for 15 min to yield protein concentrate which was washed twice and adjusted to pH 7.0, using 1M NaOH, then air-dried for 18 h and stored at 4 ºC.

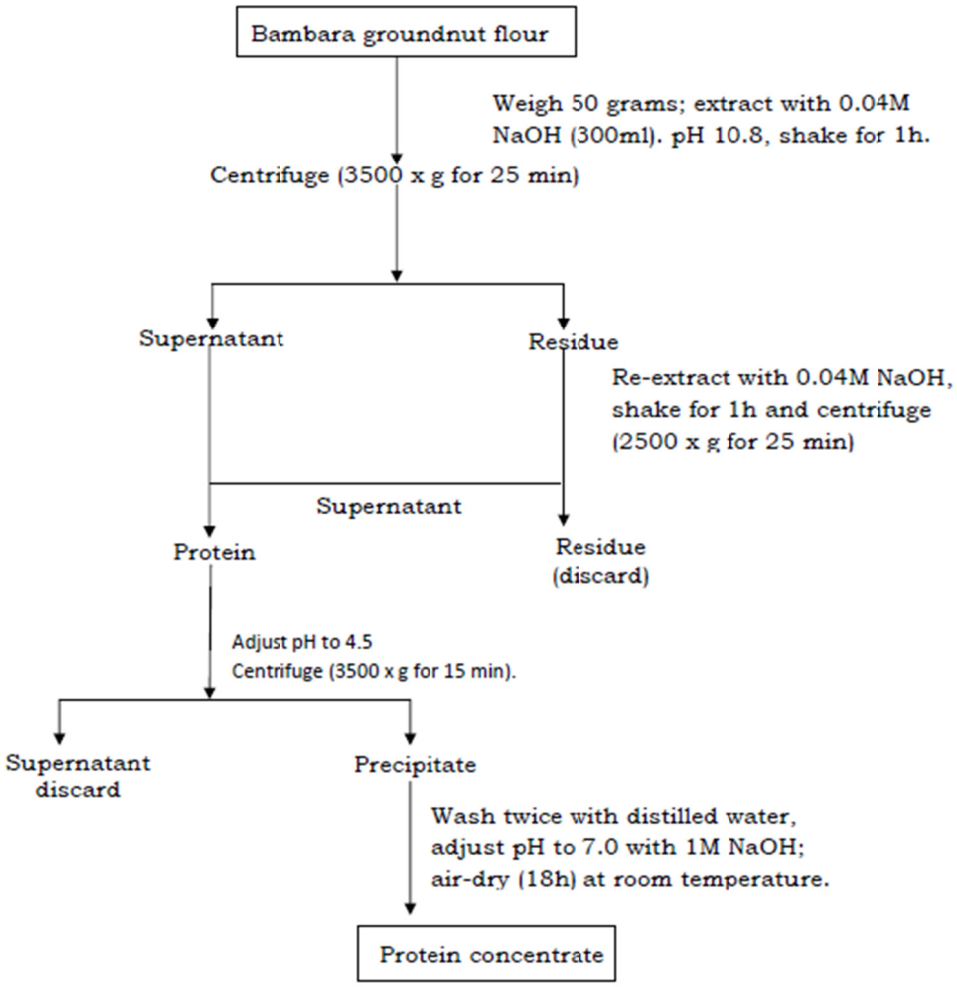

Figure 1. Flow diagrams for the production of protein concentrate from bambara groundnut flour

2.5 Preparation of Flour Blends

Graded levels of bambara groundnut protein concentrate (BGPC) ranging from 0-25% level of enrichment of plantain flour were prepared. Also, 100% wheat flour was prepared as control. The recipe is presented in Table 1.

Table 1. Recipe for the production of plantain cookies

Ingredient	Samples						
	A	B	C	D	E	F	G
Plantain flour (g)	200	190	180	170	160	150	-
BGPC (g)	-	10	20	30	40	50	-
Wheat flour (g)	-	-	-	-	-	-	200
Sugar (g)	75	75	75	75	75	75	75
Margarine (g)	125	125	125	125	125	125	125
Vanilla flavour (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Milk (powder, peak) (g)	105	105	105	105	105	105	105
Baking powder (g)	5	5	5	5	5	5	5
Egg (whole)	1	1	1	1	1	1	1

Key: Sample A: 100% PF, Sample B: 95% PF; 5% BGPC, Sample C: 90% PF; 10% BGPC,

Sample D: 85% PF; 15% BGPC, Sample E: 80% PF; 20% BGPC, Sample F: 75% PF; 25% BGPC

Sample G: 100% WF:

Where PF = Plantain flour, WF = Wheat flour, BGPC = Bambara groundnut protein concentrate.

2.6 Production of Plantain Cookies

The method described by Arisa et al. (2013) was used in the production of plantain cookies. Sugar (75 g) was added to 125 g of margarine in a Kenwood mixer and mixed at medium speed until fluffy. Whole egg and milk powder were added during mixing and then mixing continued for about 30 min. Sifted plantain flour, baking powder and flavor were slowly added to the mixture; and kneaded to form a dough. It was then rolled on a flat rolling board sprinkled with flour to a uniform thickness of about 0.4 cm, circular cookies of 5.8-6.0 cm were cut, placed in oiled baking trays and baked in the oven at 160 ºC for 15 min. Other samples with graded levels of protein concentrates and the control with 100% wheat flour were baked in the same manner.

2.7 Physico-Chemical Analysis of Plantain Cookies

The physical properties of enriched cookies were measured using the method of Zoulias et al. (2002) and reported by Giami and Barber (2004) for fluted pumpkin cookies. Physical characteristics such as weight, height, hardness, diameter and the spread ratio were calculated. The determination of the chemical composition of the cookie samples viz; moisture content, ash, protein, fat and crude fibre were determined by using AOAC method (AOAC, 2012), while carbohydrate was determined using the Clegg anthrone method as described by Osborne and Voogt (1978) and energy was calculated using the Atwater factors.

2.8 Sensory Evaluation of Enriched Cookies

Sensory evaluation of plantain cookies were carried out after baking using the method described by Giami and Barber (2004) for fluted pumpkin cookies. The sensory attribute included colour, texture, taste and general acceptability were evaluated using a 9 – point hedonic scale with 1 representing the least score (dislike extremely) and 9, the highest score (like extremely) as described by Iwe (2010).

2.9 In-vitro Protein Digestibility of Plantain Flour, Plantain Cookies

The in-vitro protein digestibility of the protein concentrates obtained from bambara groundnut flour, plantain flour (100%), non-wheat cookies enriched with bambara groundnut protein concentrates, and the control were determined using the method of Saunders et al. (1973) and modified by Monsour and Yusuf (2002). A known weight of each sample containing 16 mg nitrogen was taken and digested with 1mg of pepsin (Cat. No. P6887, Sigma Chemicals Ltd, USA) in 15 ml of 0.1 MHCl at 37 ºC for 3 h, the pepsin hydrolysed solutions were neutralized with 0.5 M NaOH and incubated with 6.0 mg of pancreatin (Sigma Chemicals Ltd, Cat. No. P 1750) in 7.5 ml of 0.2 m phosphate buffer at pH 7.6 for 18 h. The reaction was terminated by adding 22.5 ml of 10% TCA. The TCA soluble fraction was assayed for nitrogen using the micro Kjeldahl method (Foss Teactor 2001). A blank sample was also determined, protein digestibility was calculated using the formula:

$$\text{Protein digestibility \%} = \frac{\text{Nitrogen in supernatant - Nitrogen in blank}}{\text{Nitrogen in Sample}} \times 100$$

2.10 Statistical Analysis

The data obtained were analysed using the analysis of variance (ANOVA) and where means were significant, were separated using the Duncan multiple range test at the level of $P \leq 0.05$ (Wahwua, 1999).

3. Results and Discussion

The physical characteristics of cookie samples prepared from plantain flour enriched with bambara groundnut protein concentrates are presented in Table 2. The weight of cookie samples ranged from 14.7g (sample A) to 12.4 g (sample C). There was no significant difference ($P \leq 0.05$) between sample G (100% wheat flour) and samples C, D and B. However, a significant difference ($P \leq 0.05$) was observed in samples E, and F, when compared to others. The height and diameter of plantain cookies were also observed to increase gradually with increase in the level of bambara groundnut protein concentrates, up to sample D. Therefore, cookies prepared from 15% bambara groundnut protein concentrate and 85% plantain flour compared favourably in height, diameter and hardness with the control (100% wheat flour). This observation is similar to the report of Giami and Barber (2004) for cookies made from a blend of wheat flour and fluted pumpkin seed protein concentrates. Also these findings are in agreement with the work of Chinma et al. (2012), who reported the addition of defatted sesame flour to unripe plantain flour in the production of composite cookies.

Table 2. Physical characteristics of enriched cookies with bambara groundnut protein concentrate

Samples	Hardness (N)	Weight (g)	Diameter D (cm)	Height H (cm)	Spread Ratio (D/H)
A	59.1± 0.2^{a}	14.7 ± 0.3^{a}	7.6 ± 1.2^{c}	1.3± 0.01^{b}	5.9±0.5^{a}
B	58.4± 0.6^{a}	12.8 ± 1.0^{b}	7.8 ± 0.9^{c}	1.4± 0.03^{b}	5.8±0.4^{a}
C	58.6 ± 0.2^{a}	12.4 ± 0.6^{b}	8.4 ± 0.7^{b}	1.6± 0.04^{a}	5.3±0.4^{b}
D	57.9 ± 0.3^{a}	12.9 ± 0.4^{b}	9.8 ± 1.1^{a}	1.6± 0.03^{a}	6.1±0.2^{a}
E	50.4 ± 0.2^{b}	13.4 ± 1.1^{a}	9.2 ± 0.9^{a}	1.4± 0.02^{b}	6.6±0.6^{a}
F	47.5 ± 0.5^{b}	14.7 ± 0.3^{a}	8.4 ± 0.5^{b}	1.4± 0.05^{b}	6.0±0.5^{a}
G	57.8 ± 0.3^{a}	12.6 ± 0.7^{b}	10.6 ±1.2^{a}	1.7 ± 0.5^{a}	6.2 ± 0.5^{a}

^{a,b,c} Means bearing the same superscript within the same column do not differ significantly. ($P \leq 0.05$)

"±" The values are mean ± standard deviations of triplicate determinations.

Sample A = 100% PF, Sample B = 95% PF; 5% BGPC, Sample C = 90% PF; 10% BGPC,

Sample D = 85% PF; 15% BGPC, Sample E = 80% PF; 20% BGPC, Sample F = 75% PF; 25% BGPC,

Sample G = 100% WF

Where PF = Plantain flour, WF = Wheat flour, BGPC = Bambara groundnut protein concentrate.

Crude protein in the enriched cookie samples increased from 2.01% obtained in sample A (100% plantain flour) to 17.8% in sample F with 25% bambara groundnut protein concentrate is shown in Table 3. The results showed that protein concentrates improved the crude protein of cookies prepared from plantain flour. This is in agreement with the work of Arisa et al. (2013), they reported a crude protein of 4.31% for blended plantain flour and 12.5% wheat flour cookies.

It therefore followed that only 10% bambara groundnut protein concentrate would be needed to produce cookies with a crude protein content of 10.7% which compared favourably with the control (11.2%). There was no significant difference ($P \leq 0.05$) in moisture content of all samples prepared (5.4% to 6.8%). The ash and crude fibre of the enriched cookies increased from 1.21% (100% plantain flour) to 2.8% (sample F) and 5.2% to 9.2%, respectively.

The energy content of the samples ranged from 386.4 kcal/100 g (control) to 469.8 kcal/100 g in 100% plantain flour. Moisture content, crude fibre and ash values of 7.24%, 5.73% and 2.95%, respectively had been reported by Ndife et al. (2014) for cookies prepared from 50% soy flour supplemented with wheat flour.

Table 3. Proximate composition of cookies enriched with bambara groundnut protein concentrates

Samples	Crude protein (%)	Carbohy-drate (%)	Moisture content (%)	Fat (%)	Ash (%)	Crude fibre (%)	Energy (Kcal/100g)
A	2.0^{c}	60.6^{a}	5.8^{a}	25.2^{a}	1.2^{c}	5.2^{a}	469.8^{a}
B	4.8^{c}	58.2^{a}	5.8^{a}	22.6^{b}	2.2^{b}	6.4^{c}	448.1^{b}
C	10.7^{b}	53.6^{b}	5.4^{a}	21.4^{b}	2.4^{b}	6.5^{c}	443.8^{ab}
D	14.6^{b}	48.3^{c}	6.1^{a}	23.4^{b}	2.2^{b}	5.4^{d}	458.6^{a}
E	16.2^{a}	44.5^{c}	6.2^{a}	22.7^{b}	2.6^{a}	7.8^{b}	444.4^{b}
F	17.8^{a}	42.7^{c}	5.9^{a}	21.6^{b}	2.8^{a}	9.2^{a}	434.0^{c}
G (control)	11.2^{b}	47.4^{c}	6.8^{a}	18.4^{c}	1.3^{c}	7.4^{b}	386.4^{d}

Means bearing the same superscript within the same column are not significantly different.

Sample A = 100% PF, Sample B = 95% PF; 5% BGPC, Sample C = 90% PF; 10% BGPC,

Sample D = 85% PF; 15% BGPC, Sample E = 80% PF; 20% BGPC, Sample F = 75% PF; 25% BGPC,

Sample G = 100% WF; (Control).

Where WF = Wheat flour, PF = Plantain flour, BGPC = Bambara groundnut protein concentrate.

Cookies prepared using 15% bambara groundnut protein concentrate and 85% plantain flour (sample D) was acceptable with reference to colour and general acceptability which showed no significant difference ($P \leq 0.05$) when compared to the control (100% wheat flour) as shown in Table 4. This may be due to better processing control which include temperature and time regulation and efficient heat transfer in the oven which helped to prevent colour darkening that is attributed to caramelization and Maillard reactions as reported by Alobo (2001). There was no significant difference ($P \leq 0.05$) in flavor among samples D, C and F when compared to sample G (100% wheat flour). This may be due to the incorporation of protein concentrates which eliminated the beany flavor associated with bambara groundnut (Barimalaa et al., 2005) and other food legumes (Okoye and Okaka, 2009). However, this observation was contrary to the work of Arisa et al., (2013) who reported a significant difference in flavour of plantain biscuits compared with 100% wheat biscuit. The texture (hardness) of the enriched cookies decreased from a mean sensory score of 8.1 (sample G) to 6.1 (sample F). Cookies prepared from 10% bambara groundnut protein concentrate and 90% plantain flour (sample C) was not significantly different ($P \leq 0.05$) when compared with 100% wheat flour (sample G). Ndife et al. (2014) had also reported a score of 8.10 for texture for cookie samples with 30% soy flour substitution with wheat flour.

Table 4. Sensory scores for cookies enriched with bambara groundnut protein concentrate

Samples	Colour	Texture	Flavour	Overall Acceptability
A	7.4^{b}	6.5^{c}	7.6^{b}	7.3^{c}
B	7.6^{b}	7.0^{b}	7.8^{b}	7.4^{c}
C	8.4^{a}	7.7^{a}	8.1^{a}	7.7^{a}
D	8.1^{ab}	7.2^{b}	8.3^{a}	7.8^{a}
E	7.5^{b}	6.4^{c}	7.8^{b}	7.5^{b}
F	7.2^{b}	6.1^{c}	8.1^{a}	7.2^{c}
G	8.6^{a}	8.1^{a}	8.5^{a}	8.0^{a}

^{a,b,c} Means bearing the same superscripts within the same column do not differ significantly. ($P \leq 0.05$).

Sample A = 100% PF, Sample B = 95% PF; 5% BGPC, Sample C = 90% PF; 10% BGPC,

Sample D = 85% PF; 15% BGPC, Sample E = 80% PF; 20% BGPC, Sample F = 75% PF; 25% BGPC,

Sample G = 100% WF; (Control).

Where WF = Wheat flour, PF = Plantain flour, BGPC = Bambara groundnut protein concentrate.

In-vitro protein digestibilities of samples are presented in Figure 2. The protein digestibility of pure protein concentrate obtained from bambara groundnut flour was 81.82%, which was significantly higher than all other products. This value was similar to 78%, 81% and 84% protein digestibility for protein concentrate obtained from yellow millet, white millet and soybeans, respectively as reported by Tabita et al. (2009). The high values observed may be due to soluble protein fraction such as albumin and globulin which are easily hydrolyzed by enzymes. In-vitro protein digestibility increased significantly ($P \leq 0.05$) with increase in protein concentrate added to the plantain flour used for the preparation of non-wheat cookies. Digestibility increased from 27.42% (100% plantain flour) to 62.81% in sample F (25% protein concentrate and 75% plantain flour). However, samples D, E and F compared favourably with the control (100% wheat flour). The plantain cookies developed and may be beneficial for the management of metabolic disorders since unripe plantain had been reported to contain small concentration of free sugars and rapidly digestible starch (Ramandath et al., 2004).

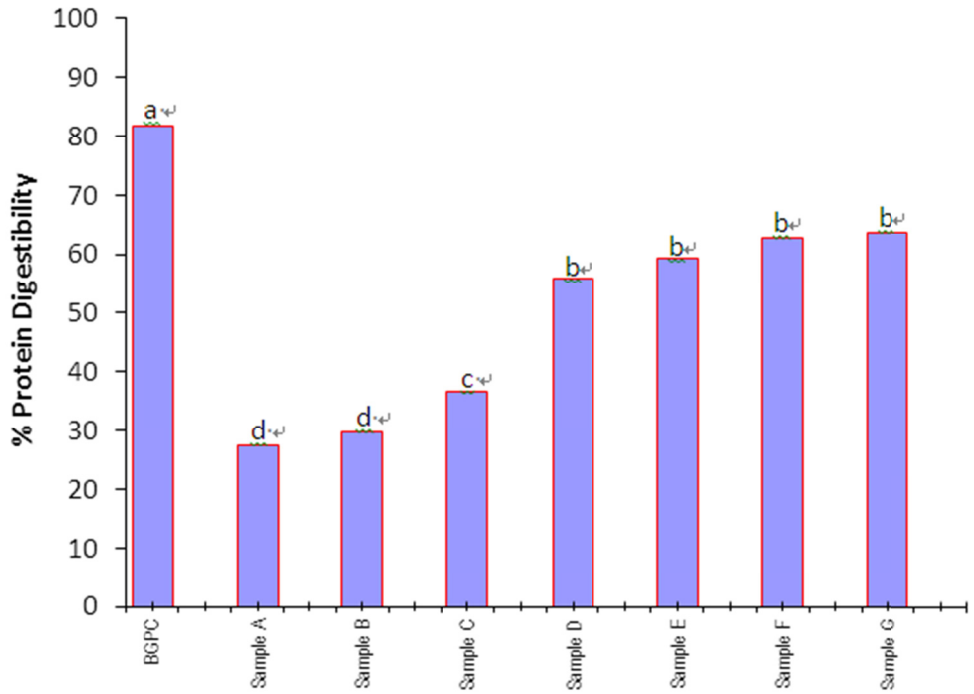

Figure 2. Results of In-vitro protein digestibility of Plantain Cookies Enriched with BGPC. Bars bearing different letters are significantly different ($P \leq 0.05$)

Sample A = 100% Plantain flour, Sample B = 95% Plantain flour + 5% BGPC,

Sample C = 90% Plantain flour + 10% BGPC, Sample D = 85% Plantain flour + 15% BGPC,

Sample E = 80% Plantain flour + 20% BGPC, Sample F = 75% Plantain flour + 25% BGPC,

Sample G = 100% Wheat flour, BGPC = Bambara groundnut protein concentrate.

4. Conclusion

Addition of bambara groundnut protein concentrate significantly improved the protein content, ash, crude fibre and energy of plantain cookies better than cookie samples with 100% wheat flour. Cookies prepared from 15% bambara groundnut protein concentrate and 85% plantain flour compared favourably in physical characteristics

with the control. Sensory scores also showed that sample D was preferred with reference to colour, flavor and general acceptability. However, in-vitro protein digestibility of cookies increased with increase in the level of protein concentrate added.

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