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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Nutritional and Sensory Quality of Cookies Supplemented with Defatted Pumpkin (*Cucurbita pepo*) Seed Flour

A.C. Atuonwu and E.N.T. Akobundu
Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

Abstract: Pumpkin (*Cucurbita pepo*) seed was processed into defatted flour (DCPF) and evaluated for nutritional and sensory attributes. The potential of the flour as composite with wheat flour in cookie production was also evaluated. The crude protein content of DCPF was as high as 57.50% with highly valuable amino acid profile, rich in essential amino acids and minerals. DCPF was highly digestible (77.91%) and has a Protein Efficiency Ratio (PER) of 1.80. The anti-nutrients were below allowable limits. Cookie diameter negatively correlated with alkaline water retention capacity of *Cucurbita pepo* seed/wheat flour blends with correlation coefficient of -0.89. The physico-chemical and sensory evaluation of cookies revealed that up to 10% substitution of wheat flour with DCPF produced acceptable cookies similar to the control (100% wheat flour).

Key words: Pumpkin seed, defatted flour, amino acid, biological value, cookies

INTRODUCTION

Ways of expanding the use of available local food sources are increasingly pursued but knowledge of the nutritive value of such local ingredients and foodstuffs is necessary in order to encourage the increased cultivation and consumption. Knowledge of the nutritive value is essential in supplementing staple carbohydrate foods. Worldwide, much research has focused on various sources of plant proteins (El-Adawy *et al*., 2001; Rangel *et al*., 2003) that may help in increasing the nutritional value of food products at low cost. Pumpkin (*Cucurbita pepo)* has received considerable attention in recent years because of the nutritional and health protective values of the seeds. The seed is an excellent source of protein and also has pharmacological activities such as anti-diabetic (Quanhong *et al*., 2003), antifungal (Wang and Ng, 2003), antibacterial and anti-inflammation activities (Caili *et al*., 2006) and antioxidant effects (Nkosi *et al*., 2006). In addition to good health benefits, pumpkin seeds are less expensive and are widely distributed. The present study examined the chemical, nutritional and supplementary potential of defatted pumpkin seed flour in biscuit making.

MATERIALS AND METHODS

Defatted *Cucurbita pepo* seed flour production: Pumpkin seeds were extracted, washed, sundried and manually decorticated. The seeds were crushed using a household mill (Super intermet blender Sl-462 model) and defatted by soaking in n-hexane for 36h with change of solvent every 8 h. The defatted flour was filtered, dried at room temperature (27°C±1°C) and ground to pass through a 355MICS sieve. The flour was packaged in an air-tight plastic container and kept in a refrigerator until analyzed.

Chemical analysis: Crude protein, fibre, moisture and vitamin C were determined by methods described by AOAC (1990). Fat, ash and mineral content were determined as described by James (1995). Carbohydrate was determined by difference and calorific value was obtained using the method of Onyeike *et al.* (1995). Thiamin and riboflavin were determined as described by Onwuka (2005). Vitamin A was determined using the method described by Martin and Ruberte (1976). Tannin, phytic acid and trypsin inhibitor were determined by the methods of Pearson (1976); Hang and Lantzsch (1983) and Arntifield *et al.* (1985), respectively. Saponin was determined by the method of Harborne (1973) and cyanogenic glycoside as described by Onwuka (2005). The oligosaccharides (stachyose and raffinose) were determined by the method of Ojiako and Akubugwo (1997).

Amino acids analysis: Amino acids were determined using a Technicon Sequential Multi-Sample Analyzer (TSM) according to the method of Speckman *et al.* (1958). *In-vitro* protein digestibility was determined using trypsin-pepsin enzyme system according to the method of Saunders *et al.* (1973). *In-vitro* digestibility

Corresponding Author: A.C. Atuonwu, Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

was expressed as percentage enzymatic digestion as shown below:

$$\text{Enzymatic digestion (\%)} = \frac{\text{Nitrogen released by enzyme}}{\text{Total nitrogen content of undigested sample}}$$

Protein Digestibility Corrected Amino Acid Score (PDCAAS) was determined using the method of Henley and Kuster (1974).

Biological values of defatted *Cucurbita pepo* seed flour was determined on the basis of the amino acid profiles. Amino acid score was calculated for each essential amino acid in a given test protein using the (FAO/WHO, 1990) reference pattern:

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g reference}}$$

The method described by Rasco (2002) was used in calculating the Essential Amino Acid Index of protein in the flour using the amino acid composition of whole egg protein as standard (Hidvégi and Békes, 1984).

$$\text{EAA I (\%)} = 100 \times \sqrt[10]{\frac{a_i}{a_{i\ ref}}}$$

Where a_i and $a_{i\ ref}$ represent the concentration of essential amino acids (lysine, tryptophan, isoleucine, valine, arginine, threonine, leucine, phenylalanine, histidine and the sum of methionine and cystine) in test sample and the reference - the egg protein, respectively.

Protein Efficiency Ratio (PER) was estimated according to the regression equation proposed by Alsmeyer *et al.* (1974).

$$\text{PER} = -0.468 + 0.454\ (\text{Leucine}) - 0.105\ (\text{Tyrosine})$$

Flour blend formulation: Defatted pumpkin seed-wheat flour blends were prepared by replacing wheat flour at 10, 20, 30 and 40% by weight.

Alkaline water retention capacity: The method described by Sathe *et al.* (1982) was used to evaluate the alkaline water retention capacity of the flour blends.

Preparation of cookies: The recipe used for cookie preparation included flour 60g, vegetable shortening 50g, granulated sugar 25g, baking powder 0.6g, nutmeg 200 mg, salt 700 mg, and water 5 ml. Cookies were prepared by replacing the all purpose wheat flour with *Cucurbita pepo* seed flour at 10, 20, 30, and 40 % (by weight). The dough was allowed to equilibrate for 1 h at 4°C and divided into 15 g portions, shaped round and baked on a greased tray at 149°C for 25 min. Baked cookies were cooled to room temperature and evaluated for physical parameters and sensory properties (Sathe *et al.*, 1982).

Plate 1: Cookies from 0:100 DCPF-wheat flour blend

Plate 2: Cookies from 10:90 DCPF-wheat flour blend

Plate 3: Cookies from 20-80 DCPF-wheat flour blend

Physical evaluation of cookies: Weight, height and diameter measurements were performed in triplicate on

Plate 4: Cookies from 30:70 DCPF-wheat flour blend

Plate 5: Cookies from 40:60 DCPF-wheat flour blend

Plate 6: Cookies from 100:0 DCPF-wheat flour blend

five representative cookies in each batch. The cookie spread ratio (%) was calculated as the increase in volume of the unbaked stamped out dough:

$$\text{Spread ratio (\%)} = \frac{\text{Increase in volume of cookie dough}}{\text{Original volume of cookie dough}} \times 100$$

Cookie break strength was determined (Okaka and Isieh, 1990).

Sensory evaluation: Twenty untrained judges comprising of staff and students of Michael Okpara University of Agriculture, Umudike were used for the evaluation of the quality parameters (colour, taste, texture, flavor and general acceptability) of the cookies. The panelist were asked to indicate their preference using a nine-point Hedonic scale with 1 and 9 representing liked extremely and disliked extremely, respectively.

Statistical analysis: Correlation analysis was performed on duplicate determination for alkaline water retention capacity and the data obtained from sensory evaluation were subjected to Analysis of Variance (ANOVA) the SPSS statistical package (Version 13.0). Means were separated with Duncan's Multiple Range Test (DMRT). Significant differences were determined at $p<0.05$ level and results were expressed as the mean value ± standard deviation of duplicate determinations.

Table 1: Anti-nutritional factors in pumpkin flour

Tannin (%)	0.69
Saponin (%)	0.56
Hydrogen cyanide (mg/100 g)	4.08
Trypsin Inhibitor (TIU/g)	2.07
Phytate (%)	0.44
Stachyose (%)	3.00
Raffinose (%)	0.80

RESULTS AND DISCUSSION

Nutritional properties: Hydrogen cyanide was found to be 4.08 mg (Table 1). HCN values from this study were below the safety level for cyanide poisoning in man. The lethal dose range of ingested HCN for humans is estimated to be 50-60 mg/kg body weight/day as reported by Balagopalan *et al.* (1988). Stachyose and raffinose were high compared to Udensi *et al.* (2008) who recorded lower values for stachyose (1.29%) and raffinose (0.32%) in *Mucuna flagellipes*. Raffinose and stachyose have been identified as flatulence inducers and when ingested cause accumulation of gas, discomfort, diarrhea, pain and cramps (Liew and Buckle, 1990), a factor which tends to render legumes less acceptable.

Amino acid: The amino acid composition of *Cucurbita pepo* seed flour is presented in Table 2. *Cucurbita pepo* seed flour exhibited lower amino acid content compared to chickpea flour which ranged between 1.6-19.5 g/100 g protein (El-Adawy *et al.*, 2001). Cystine and tryptophan showed the lowest values 0.79 and 0.99 g/100 g protein,

Table 2: Amino acid profile of pumpkin (*Cucurbita pepo*) seed flour

Amino acid	Composition g/100 protein	FAO / WHO / UNU (1985) pre-school child (2-5 yrs) reference pattern (g/100 g protein)	Uncorrected amino acid score	PDCAAS
Isoleucine	2.98	2.80	1.06	0.82
Leucine	5.71	6.60	0.87	0.67
Lysine	4.30	5.80	0.74	0.58
Cystine[a]	0.79	-	-	-
Methionine[a]	1.43	Methionine + cystine = 2.50	0.89	0.69
Total sulphur amino acid	2.22	-	-	-
Tyrosine	3.06	-	-	-
Phenylalanine	3.47	Phenylalanine + tyrosine =6.30	1.04	0.81
Total aromatic amino acids	6.53	-	-	-
Threonine	2.11	3.40	0.62	0.48[a]
Tryptophan[a]	0.99	1.10	0.90	0.70
Valine	4.50	3.50	1.29	1.01
Histidine	2.33	1.90	1.23	0.96
Arginine	4.85			
Aspartic acid	8.91	-	-	-
Glutamic acid	9.50	-	-	-
Serine	2.19	-	-	-
Proline	2.12	-	-	-
Glycine	3.80	-	-	-
Alanine	4.76	-	-	-
E/T (%)	43.27	-	-	-

[a] = Limiting Amino Acid, E/T = Essential to Total Amino Acid, PDCAAS = Protein Digestibility Corrected Amino Acid Score

Table 3: Protein nutritional quality of *Cucurbita pepo* seed flour

Samples	Chemical score (%)	Limiting amino acids First	Second	Third	EAAI (%)	*In-vitro* protein digestibility (%)	PER
DCPF	62.0	Threonine	Lysine	Leucine	57.31	77.91	1.80

DCPF = *Cucurbita pepo* Seed Flour; EAAI = Essential Amino Acid Index; PER = Protein Efficiency Ratio

respectively. Percentage ratios of essential to total amino acids (E/T, %) for *Cucurbita pepo* seed flour (43.27) was above 36% which is considered adequate for an ideal protein (FAO/WHO, 1973). The protein nutritional quality of *Cucurbita pepo* seed flour is presented in Table 3. The first, second, and third limiting amino acids were threonine, lysine and leucine, respectively. The present observation is similar to other legumes (Sathe *et al.*, 1982; Akobundu *et al.*, 1982). The *in-vitro* protein digestibility exhibited by the flour was high and may be attributed to its low fat and protease inhibitor content which usually hinder the action of digestive enzymes when present in large amount (Sánchez-Vioque *et al.*, 1999). It could also be attributed to the low tannin content (Table 1). Tannins cause reduction in digestibility of dietary protein (Barroga *et al.*, 1985).

Alkaline water retention capacity of *Cucurbita pepo* seed composite flour: The alkaline water retention capacity predicts the baking behaviour of flour in cookies based on the established inverse relationship between alkaline water retention and baked cookie diameter. The cookie diameter was negatively correlated to the alkaline water retention capacity of the blends (Table 4) with correlation coefficient of -0.89. Similar correlation between alkaline water retention capacity and cookie diameter for several wheat flours and Great Northern Bean (*Phaseolus vulgaris L.*) were reported by Yamazaki *et al.* (1977) and Sathe *et al.* (1982), respectively. Twenty percent wheat flour substitution had the highest alkaline water retention capacity with reduced cookie diameter while 100% wheat flour gave the least alkaline water retention with high cookie diameter (Table 4).

Table 4: Correlation between the alkaline water retention capacity of defatted *Cucurbita pepo* seed flour blends and cookie diameter

DCPF-wheat flour blends	Alkaline water retention capacity[a] (g/g blend)	Cookie diameter[a] (cm)	Correlation coefficient
cpw (control)	0.95±0.28	6.22±0.01	
cpw_1	1.05±0.35	6.21±0.06	
cpw_2	1.18±0.18	6.19±0.01	-0.89
cpw_3	1.00±0.21	6.20±0.03	
cpw_4	1.15±0.07	6.15±0.07	

[a]Mean±standard deviation of duplicate determinations.
cpw_1= Defatted *Cucurbita pepo* seed-wheat flour (10:90)
cpw_3= Defatted *Cucurbita pepo* seed-wheat flour (30:70)
cpw (control) = Defatted *Cucurbita pepo* seed-wheat flour (0:100)
cpw_2= Defatted *Cucurbita pepo* seed-wheat flour (20:80)
cpw_4= Defatted *Cucurbita pepo* seed-wheat flour (40:60)

Physicochemical properties of cookies: Physical parameters and protein content of cookies are

Table 5: Physicochemical properties of cookies

DCPF-wheat flour blends	Protein content (%)	Increase in protein content[a] (%)	Weight (g)	Diameter (cm)	Height (cm)	Spread ratio (%)	Break strength (Kg)
cpw (control)	6.80	0.00	13.30	6.22	0.60	66.03	0.46
cpw_1	6.92	1.76	13.39	6.21	0.53	51.22	0.46
cpw_2	7.00	2.94	13.34	6.19	0.53	46.83	0.51
cpw_3	8.59	26.32	13.31	6.20	0.53	47.29	0.68
cpw_4	10.00	47.06	13.53	6.15	0.47	28.52	0.62

[a]Increase (%) with reference to protein content of the control cookies. cpw (control) = Defatted *Cucurbita pepo* seed-wheat flour (0:100)
cpw_1= Defatted *Cucurbita pepo* seed-wheat flour (10:90) cpw_2= Defatted *Cucurbita pepo* seed-wheat flour (20:80)
cpw_3= Defatted *Cucurbita pepo* seed-wheat flour (30:70) cpw_4= Defatted *Cucurbita pepo* seed-wheat flour (40:60)

Table 6: Sensory evaluation[e] of cookies made from DCPF-wheat flour blends

DCPF-wheat flour blend	Colour	Taste	Texture	Flavour	General acceptability
cpw (control)	1.65^{a}±0.7452	2.30^{ab}±0.4702	2.45^{ab}±0.6863	1.90^{a}±0.7182	2.20^{ab}±0.6156
cpw_1	2.10^{ab}±0.5525	2.05^{a}±0.5104	2.15^{a}±0.8127	2.10^{a}±0.7182	1.90^{a}±0.7881
cpw_2	2.25^{ab}±0.7164	3.05^{c}±1.0990	2.95^{b}±0.6863	2.80^{b}±0.7678	3.00^{c}±1.0260
cpw_3	2.20^{ab}±0.8944	2.80^{bc}±0.7678	2.50^{ab}±0.6883	2.90^{b}±1.0208	2.90^{bc}±0.7182
cpw_4	2.60^{b}±1.0955	3.30^{c}±0.9234	2.95^{b}±1.1459	3.75^{c}±1.3328	3.05^{c}±1.3169

[e]Mean± standard deviation of duplicate determinations.
[a,b,c,d]Means with the same superscripts within the same column are not significantly different ($p<0.05$).
cpw (control) = Defatted *Cucurbita pepo* seed-wheat flour (0:100) cpw_1= Defatted *Cucurbita pepo* seed-wheat flour (10:90)
cpw_2= Defatted *Cucurbita pepo* seed-wheat flour (20:80) cpw_3= Defatted *Cucurbita pepo* seed-wheat flour (30:70)
cpw_4= Defatted *Cucurbita pepo* seed-wheat flour (40:60)

presented in Table 5. Increase in wheat flour substitution with *Cucurbita pepo* seed flour increased the protein content of the cookies. Generally, the cookie spread ratio decreased with increase in protein content of the cookies. It was reported that rapid partitioning of free water to hydrophilic sites during mixing increased dough viscosity and limited cookie spread ratio during baking (McWatters, 1978). The reduction in spread ratio was more pronounced in 40% wheat flour substitution. Similar results were recorded by Sathe *et al.* (1982) for cookies made with Northern Bean (*Phaseolus vulgaris L.*) flour. The break strength of the cookies increased with increase in protein content. *Cucurbita pepo* seed flour-wheat blend of 30% had the highest break strength while 10% wheat flour substitution emerged the least and had the same value as 0% substitution (Control).

Sensory properties of cookies: The sensory evaluation of cookies made from *Cucurbita pepo* seed-wheat flour blends is presented in Table 6. The cookies were not significantly different in colour at 10-30% wheat flour substitution. There was no significant difference in texture at all levels of wheat flour substitution. Apart from the control, cookies made from 10% wheat flour substitution had the best flavour. This result revealed that up to 10% substitution of wheat flour with defatted *cucurbita pepo* seed flour produced acceptable cookies which were not significantly different from the control (100% wheat flour).

Conclusion: In addition to good chemical and nutritional values, *Cucurbita pepo* seed flour performed well as composite in cookie production. Wheat flour substitution at 10% is recommended to produce acceptable cookies. Observations in this study further support high correlation between alkaline water retention capacity and cookie diameter and that *Cucurbita pepo* seed flour has the potential of being used as a nutritional supplement.

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