

**Fig 2: Flow Chart for the Production of bread fortified Pumpkin seed Milk Bread**

Source: Modified Sanful and Darko, (2010)

## Methods

All analyses are carried out in duplicates and all the calculations for the different parameters determined under this section are done as shown in appendix.

## Bread Physical Characteristics Evaluation

Bread characteristics were evaluated by measuring the loaf weight, loaf volume and specific loaf volume.

**Loaf weight:** Loaf weight was measured 30 minutes after the loaves were removed from the oven using a weighing balance

**Loaf Volume:** loaf Volume was measured using the rapeseed displacement method as modified by Giamiet *al.*, 2004 as follows: A box of fixed dimensions (23.00 x 14.30 x 17.21 cm) of internal volume 5660.37 [cm.sup.3] was put in a tray, half filled with pearled millet, shaken vigorously 4 times, then filled till slightly overfilled so that overspill fell into the tray. The box was shaken again twice, and then a straight edge was used to press across the top of the box once to give a level surface. The seeds were decanted from the box into a receptacle and weighed. The procedure was repeated

three times and the mean value for seed weight was noted (C g). A weighed loaf was placed in the box and weighed seeds (3500 g) were used to fill the box and leveled off as before. The overspill was weighed and from the weight obtained the weight of seeds around the loaf and volume of seed displaced by the loaf were calculated using the following equations by AACC method 10-05.01(AACC 2000):

Seeds displaced by loaf (L) = C g + overspill weight - 3500 g..... (1)

Volume of loaf (V) = L x 5660.37 [cm.sup.3] / C

**Specific Loaf Volume** was determined by dividing the loaf volume by its corresponding loaf weight ([cm.sup.3] /g) as described by Araki *et al.*, 2009

**Loaf Diameter** was determined using veneer caliper

**Loaf Height** was determined using meter rule

### **Proximate Composition**

The flour samples were subjected to proximate analysis. The proximate analysis was done to obtain values for the moisture content, dry matter, crude protein, crude fibre, crude fat, and ash content following the procedures described by AOAC (2010).

#### **Determination of Moisture Content**

The moisture content is determined by the method of AOAC (2010). Cleaned crucibles is dried in the oven at 100°C for 1hr to obtain a constant weight and then cooled in the desiccators. Two gram of the samples is weighed into the crucible and dried at 100°C until a constant weight will be obtained.

#### **Determination of Ash Content**

The AOAC (2010) method is used to determine the ash content. Two grams of sample is weighed into a pre-heated cooled crucible. This sample is charred on a Bunsen flame inside a fume cupboard. Sample will be transferred into a pre-heated muffle furnace at 550°C for 3-5hrs until a white or light grey ash is obtained.

#### **Determination of Crude Protein Content**

The protein content of the samples will be determined according to the standard method of AOAC (2010) using the Kjeldal method described below.

**Digestion of sample:** Two grams of sample will be weighed in a kjeldal flask. About 5g anhydrous sodium sulphate or 2 tablets of kjedahl catalyst will be added. Twenty five millimeter (25mm) Conc. H<sub>2</sub>SO<sub>4</sub> will be added with few boiling chips (antibumps). It will be heated in the fume chamber until the solution becomes clear. The solution is then cooled to room temperature after which it is transferred into a 250ml volumetric flask and made up to the level with distilled water.

**Distillation:** A 100ml conical flask, containing 5ml of 2% boric acid will be placed under the condenser with addition of drops of methyl red indicator. Five milliliter (5ml) of the digest is pipette into the apparatus through the small funnel washed down with distilled water followed by addition of 5ml of 60% NaOH solution. The solution in the receiving flask is titrated with 0.049 H<sub>2</sub>SO<sub>4</sub> to get a pink colour.

#### **Determination of Fat Content**

The Soxhlet extraction method of AOAC (2010) will be used. A Soxhlet extractor with a reflux condenser and a 500ml round bottom flask is fixed. 2g sample will be weighed into a labeled thimble. Petroleum ether (300ml) will be filled into the round bottom flask. The extractor thimble will be sealed with cotton wool. The Soxhlet apparatus will be allowed to reflux for 6hrs, the thimble is removed with care and the petroleum ether collected at the top and drained into a container for reuse. When the flask is free of ether, it will be removed and dried at 105°C for 1hr in an oven, cooled in desiccators and weighed.

#### **Determination of Crude Fiber Content**

The crude fiber will be determined according to the method of AOAC (2010). Petroleum ether will be used to defat 2g of sample. The defatted sample is boiled in 200ml of 1.25% H<sub>2</sub>SO<sub>4</sub> and boiled for 3 minutes, the solution will be filtered through muslin/linen cloth on a fluted funnel. It will be washed with boiling water until it is free of acid. The residue will be returned into 200ml boiling NaOH and allowed to boil for 30mins. It is further washed with 1% HCL and hot water, to free it of acid. The final residue will be drained, dried and transferred to a crucible and ignited in a muffle furnace.

#### **Determination of Carbohydrate Content**

Carbohydrate content of the samples will be determined by the difference method described by Oyenuga (1968) using the equation

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ crude fiber} + \text{crude fat}) \dots \dots \dots (2)$$

#### **Sensory Evaluation**

The prepared bread samples were presented to an untrained 15-member panel of judges made up of staff members and students of the department of Food Science and Technology, University of Agriculture, Makurdi, Benue State who are familiar with the consumption of bread. The samples were assessed for Crust appearance, Crumb color, Crust texture, Crumb texture, Flavor/Aroma, Eat ability (taste/mouth feel) and Overall acceptances using a nine-point hedonic scale, where 9 indicated “like extremely” and 1 indicated “dislike extremely”. The bread samples were sliced into pieces of uniform thickness (2 cm), coded with 3-digit random number using statistical random tables and served to the panelists at around 11.15 a.m. The panelists were instructed to rate the attributes indicating their degree of liking or disliking by putting a number as provided in the hedonic scale according to their preference. Each panelist was provided with enough water to rinse mouth in between taste and privacy to avoid biased assessment.

#### **Statistical Analysis**

All determinations were conducted in triplicate. The means and standard deviations were also calculated. Data generated was subjected to analysis of variance (ANOVA). The mean was tested using least significant difference (LSD)

### **3.0 RESULTS AND DISCUSSION**

#### **Effect of Pumpkin Milk on Physico-Chemical Composition of Bread**

The results obtained from the chemical analysis and the physical properties investigated are shown in Table 1. The increased fortifications of wheat flour with pumpkin seed milk greatly affected the physico-chemical quality of the bread. The proximate values for moisture, ash, fat, protein and carbohydrate, were lowest in whole wheat bread (sample A), which served as control and higher in other pumpkin milk fortified samples. The proximate values increased with increasing levels of



pumpkin milk fortifications except for crude fibre which showed the reverse. The crude fibre values were highest in sample A (2.40%) and lowest in sample E (2.20%), respectively. The low fibre values were as result of the low fibre content of the pumpkin seed as reported by Henriques *et al.* (2012). The carbohydrates and energy values of the samples increased significantly with increase in pumpkin milk. The high carbohydrate and energy values were as result of the high fat content of the pumpkin seed milk. Similar observations were reported by Seremet *et al.* (2011) and Islam *et al.* (2007) in the fortification of wheat flours with defatted and non-defatted soy flour, respectively. The breads contained energy values in the range of 247 to 360 Kcal, and hence conformed to the (FAO/WHO, 1994) recommended minimum energy content of 1674 kJ/ 100 g.

The increased carbohydrate and the lower fibre content of composite breads have several health benefits, as it will aid in the digestion of the bread in the colon and reduce constipation often associated with bread produced from refined wheat flour (Jideani and Onwubali, 2009; Elleuchet *et al.*, 2011). According to well documented studies, it is now accepted that dietary fibre plays a significant role in the prevention of several diseases such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes (Slavin, 2005; Elleuchet *et al.*, 2011). The crude fibre contents of the composite breads, was within the recommended range of not more than 6 g dietary fibre and other no absorbable carbohydrates per 100 g dry matter (FAO/WHO, 1994). Vitalis *et al.* (2009) reported that using whole grain raw materials and combining wheat flour with certain legumes and pseudocereals in biscuit production, resulted in improved nutritional and functional properties of the final product.

The moisture contents of the breads increased significantly ( $p \leq 0.05$ ) with pumpkin seed milk substitution by a range of 28.50 to 39.50%. This agree with findings of earlier workers (Akhtar *et al.*, 2008; Elleuchet *et al.*, 2011; Manejuet *et al.*, 2011). High moisture content has been associated with short shelf life of composite breads as they encourage microbial proliferation that lead to spoilage (Ezeama, 2007). There was also significant ( $p \leq 0.05$ ) increase in the protein content of the composite breads with pumpkin seed milk substitution in the range of 8.13 to 12.50%. This increase is as a result of substitution of pumpkin seed milk. Mashayekhet *et al.* (2008) and Sanful and Darko (2011) also reported increase in protein content of the bread as a result of the addition of soy flour. Other studies have also reported a similar increase of protein content in sorghum-soy composite flours (Singh *et al.*, 2000; Awadel-kareemet *et al.*, 2008). The increase in the protein content of the bread could be due to the significant quantity of protein in the pumpkin seeds (Kure *et al.*, 1998; Basmanet *et al.*, 2003). The high protein content in the pumpkin supplemented breads studied in this work would be of nutritional importance in many developing countries like Nigeria where many people are unable to afford foods with high protein because such foods are quite expensive.

The fat content also increased significantly ( $p \leq 0.05$ ) from 4.00 to 5.56% in the composite breads produced from pumpkin seed milk substitution. Pumpkin seed from which the milk was produced from is an oil seed, must have contributed most of the oil content to the product. The high oil content of the composite bread will affect the shelf stability (Weiss, 2000; Potter and Hotchkiss, 2006). The ash content increased with the increase in proportion of pumpkin milk in the bread. This result agrees with results of other workers (Olaoye *et al.*, 2006, Sanful and Darko, 2011). The increase in ash content could be due to the higher ash content of the pumpkin than in the wheat flour. The pumpkin seeds have been reported to contain an appreciable quantity of minerals and fat (Ariahuet *et al.*, 1999; Onyeka and Dibia, 2002; Plaharet *et al.*, 2003).

**Table 3: Effect of Pumpkin Milk on Physico-Chemical Composition of Bread**

Parameters	A	B	C	D	E	LSD
Moisture (%)	28.50±0.13 <sup>e</sup>	33.00±0.15 <sup>d</sup>	36.00±0.16 <sup>c</sup>	37.50±0.10 <sup>b</sup>	39.50±0.15 <sup>a</sup>	0.97
Protein (%)	8.13±0.23 <sup>e</sup>	9.44±0.15 <sup>d</sup>	10.78±0.35 <sup>c</sup>	11.78±0.25 <sup>b</sup>	12.50±0.20 <sup>a</sup>	0.45
Fat (%)	4.00±0.03 <sup>e</sup>	4.50±0.02 <sup>d</sup>	4.74±0.05 <sup>c</sup>	5.56±0.03 <sup>b</sup>	6.40±0.05 <sup>a</sup>	0.42
Crude fibre (%)	2.40±0.05 <sup>a</sup>	2.35±0.15 <sup>ab</sup>	2.30±0.10 <sup>b</sup>	2.24±0.15 <sup>b<sup>c</sup></sup>	2.20±0.20 <sup>c</sup>	0.59
Ash (%)	1.80±0.30 <sup>d</sup>	2.00±0.30 <sup>c</sup>	2.30±0.20 <sup>b</sup>	2.50±0.25 <sup>a</sup>	2.65±0.20 <sup>a</sup>	0.16
Carbohydrate (%)	44.83±0.15 <sup>e</sup>	51.26±0.20 <sup>d</sup>	53.82±0.15 <sup>c</sup>	59.58±0.20 <sup>b</sup>	63.25±0.25 <sup>a</sup>	1.50
Energy (Kcal)	247.84±0.35 <sup>d</sup>	283.30±0.25 <sup>c</sup>	301.06±0.30 <sup>c</sup>	335.48±0.25 <sup>b</sup>	360.60±0.35 <sup>a</sup>	24.60

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ( $p \geq 0.05$ ).

**Key:**

A = Control. B = 10% pumpkin milk. C = 20% pumpkin milk. D = 30 pumpkin milk. E = 40% pumpkin milk.

LSD = Least Significant Difference

### **Effect of Pumpkin Milk on Baking Characteristics of Bread**

Results of the physical characteristics of bread samples containing different levels of pumpkin milk substitution as compared to the control is also shown in Table 2. The bread volume increased significantly ( $p \leq 0.05$ ) by a range of 1021 cm<sup>3</sup> to 1157.36 cm<sup>3</sup>, as the level of substitution with pumpkin seed milk increased. Albert (1997) and Gomez et al. (2002) reported that, the main problem of dietary fibre addition in baking is the important reduction of loaf volume and the different texture of the breads obtained. Increased supplementation of wheat flour with defatted and non-defatted soy-flour reduced loaf volume and specific volume drastically (Constandache, 2005; Rodriguez et al., 2006; Islam *et al.*, 2007). Dietary fibre additions, in general, had pronounced effects on dough properties yielding higher water absorption, mixing tolerance and tenacity, and smaller extensibility in comparison with those obtained without fibre addition (Gomez *et al.*, 2002; Elleuchet *et al.*, 2011). The deleterious effects of addition of fiber on dough structure and loaf volume have been suggested to be due to the dilution of gluten network, which in turn impairs gas retention rather than gas production (Eimanet *et al.*, 2008; Elleuchet *et al.*, 2011). However in this study, the increased in loaf volume could be attributed to the low fibre content of the pumpkin seed milk. The same trend was observed for the density, since specific volume and density are directly related. Density of bread samples decreased significantly ( $p > 0.05$ ) with increasing proportion of pumpkin milk, ranging from 0.37 to 0.35 g/cm<sup>3</sup>. However, there was no significant difference between bread loaf density of the control and the breads made with 10 %, 20% and 30% and 40% of pumpkin seed milk. This confirms the findings of Eriksson (2013) who observed a significant increase in density of bread produced from different variety of cassava flour. The bread loaf showed a significant increase in weight, height, and diameter with increased in pumpkin seed milk. Higher loaf weight and volume are desired by bakers on bread at the addition of different substitutes. Therefore, bread weight and volume increment after adding substitutes is a desirable economic quality to the baking industries as customers often get attracted to bread loaf with higher weight and volume believing that it is more valuable for the same price (Wu *et al.*, 2009). Loaf weight is affected by the quantity of dough baked and the amount of moisture and carbon dioxide diffused out of the loaf during baking. Loaf volume is determined by the quantity and quality of protein in the flour (Othiraet *et al.*, 2004) and proofing time (Zghalet *et al.*, 2002).

**Table 4: Effect of Pumpkin Milk on Baking Characteristics of Bread**

Parameters	A	B	C	D	E	LSD
Loaf weight (g)	373.80± 1.98 <sup>b</sup>	385.83± 1.00 <sup>a</sup>	388.70 ± 1.44 <sup>a</sup>	394.99± 0.43 <sup>a</sup>	400±1.07 <sup>a</sup>	17.80
Loaf volume (cm <sup>3</sup> )	1021±0.25 <sup>b</sup>	1035.79±0.35 <sup>b</sup>	1050±0.30 <sup>a</sup>	1092.96±0.25 <sup>a</sup>	1157.36±0.35 <sup>a</sup>	107.4
Specific vol. (cm <sup>3</sup> /g)	2.73±0.10 <sup>a</sup>	2.68±0.20 <sup>a</sup>	2.70±0.15 <sup>a</sup>	2.77±0.10 <sup>a</sup>	2.89±0.15 <sup>a</sup>	0.39
Length (cm)	18.50±0.00 <sup>a</sup>	18.20±0.20 <sup>c</sup>	18.30±0.10 <sup>bc</sup>	18.40±0.10 <sup>ab</sup>	18.50±0.20 <sup>a</sup>	0.16
Height (cm)	6.00±0.10 <sup>b</sup>	6.20±0.10 <sup>ab</sup>	6.50±0.10 <sup>ab</sup>	6.60±0.10 <sup>ab</sup>	7.20±0.10 <sup>a</sup>	1.01
Diameter (cm)	9.20±0.20 <sup>a</sup>	8.50±0.20 <sup>c</sup>	9.00±0.20 <sup>b</sup>	9.10±0.20 <sup>ab</sup>	9.20±0.20 <sup>a</sup>	0.16
Density	0.37 <sup>a</sup>	0.37 <sup>a</sup>	0.37 <sup>a</sup>	0.36 <sup>ab</sup>	0.35 <sup>b</sup>	0.02

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ( $p \geq 0.05$ ).

Key:

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LSD

=

Least

Significant

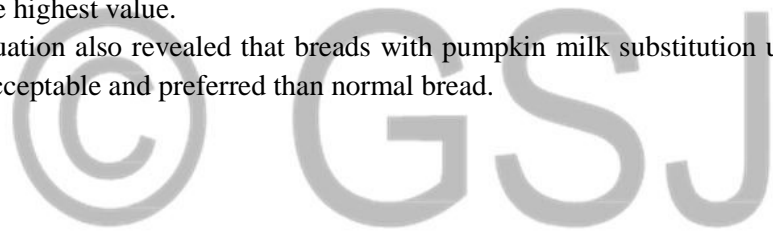
Difference

### **Sensory Attribute of Bread Sample**

Results of sensory evaluation of bread samples containing different level of pumpkin seed milk substitution as compared to the control is shown in Table 3. The results of bread crust appearance and crumb colour showed a consistent pattern for all the bread samples, and there was significant difference in the bread samples and the control sample. The darker color of the crumbs of whole wheat bread and fortified breads and biscuits have been reported by several authors (Singh *et al.*, 2000; Akhtar *et al.*, 2008; Serremet *et al.*, 2011). The brownish bread appearance could be directly related to the increase in fiber content (Hu *et al.*, 2007). Moreover browning of the breads could also occur due to caramelization and maillard reactions, as the protein contributed by pumpkin seed milk must have reacted with sugar during the baking process (Dhingra and Jood, 2001; Mohsen *et al.*, 2009).

The scores for texture (softness and chewiness) of the composite bread samples, increased with increase in pumpkin seed milk substitution, when compared to whole wheat bread (control sample A). The bread with 40% pumpkin seed milk substitution (sample E), had the best texture score. Hard crumb texture, caused by increased fiber from wheat bran substitution was reported by Eimanet *al.* (2008). The baking conditions (temperature and time variables); the state of the bread components, such as fibers, starch, protein (gluten) whether damaged or undamaged and the amounts of absorbed water during dough mixing, all contribute to the final texture of the breads (Gomez *et al.*, 2003; Bakke and Vickers, 2007; Akhtar *et al.*, 2008; Serremet *et al.*, 2011). The incorporation of pumpkin milk into wheat bread resulted in good flavour scores. The results showed an increase in the scores as the whole-wheat flour was substituted with pumpkin seed milk. Sample E with 40% pumpkin seed milk recording the highest value.

The sensory evaluation also revealed that breads with pumpkin milk substitution up to 40% (sample E) were overall acceptable and preferred than normal bread.



**Table 5: Sensory Scores of Bread Samples.**

Parameters	A	B	C	D	E	LSD
Crust appearance	6.27 <sup>d</sup>	6.80 <sup>cd</sup>	7.00 <sup>bc</sup>	7.47 <sup>b</sup>	8.13 <sup>a</sup>	0.65
Crumb colour	6.33 <sup>c</sup>	6.40 <sup>b<sup>c</sup></sup>	7.20 <sup>b</sup>	7.40 <sup>ab</sup>	7.93 <sup>a</sup>	0.63
Crumb Texture	6.33 <sup>c</sup>	6.67 <sup>bc</sup>	7.20 <sup>b</sup>	7.47 <sup>ab</sup>	8.13 <sup>a</sup>	0.71
Flavour	5.93 <sup>d</sup>	6.60 <sup>c</sup>	7.33 <sup>b</sup>	7.80 <sup>b</sup>	8.47 <sup>a</sup>	0.63
Taste	6.20 <sup>d</sup>	6.93 <sup>c</sup>	7.13 <sup>bc</sup>	7.60 <sup>b</sup>	8.47 <sup>a</sup>	0.59
Overall acceptability	5.93 <sup>c</sup>	6.40 <sup>c</sup>	7.13 <sup>b</sup>	7.73 <sup>b</sup>	8.40 <sup>a</sup>	0.64

Values are means of triplicate determinations. Mean values with same superscript in a row are not significantly different ( $p \geq 0.05$ ).

Key:

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LSD = Least Significant Difference

## 4.0 CONCLUSION AND RECOMMENDATIONS

### Conclusion

The composite breads with pumpkin seed milk substitutions were found to be nutritionally superior (have higher protein, fat and carbohydrate content) to whole wheat bread. The baking characteristics of the bread were significantly improved. Also the scores for organoleptic attributes like taste, aroma, texture (mouth feel), and colour were generally superior to that of whole-wheat bread. Therefore, the pumpkin milk bread had better overall acceptability scores than the wheat breads.

### Recommendations

From the study carried out, it is recommended that:

- Pumpkin milk should be included in daily diet plan through its incorporation into wheat flour used for production of bread.
- Baking industry should focus on the fortification of bakery products with vegetable milk such as Pumpkin seed milk to reduce over dependence on dairy milk.
- Further research work should be focused on the shelf stability of pumpkin seed milk bread

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