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Proximate Analysis of Some Bread Samples Available in Calabar, Nigeria and Some Chemical Compounds in Them Using Gas Chromatography-Mass Spectrometry (GC-MS)

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Abstract

This study aimed at carrying out proximate analysis and determining the chemical compounds present in some bread samples consumed in Calabar, Nigeria in order to ascertain their nutritional value and quality to ensure food safety and safeguard the health of people. Four popular bread samples were collected from the shops and taken to University of Calabar Chemistry Laboratory for proximate analysis and their extract were also analyzed for some compounds using GC-MS. The results proximate analysis revealed the mean values to range between (4.46 - 6.39)% moisture, (2.78 – 8.89)% ash, (0.35 – 3.34)% fibre, (3.40 – 8.46)% protein, (1.63 – 4.17)% fat/lipid and (77.44 – 85.29)% carbohydrate. The GC-MS result revealed a lot of chemical compounds but the notable ones considered were: Hexadecane-1.13%, Octadecane-0.56%, Nonadecane-0.72%, 9-Eicosene-2.20%, 2,4-Di-tert-butylphenol-0.30%, Dodecanoic acid-1.80%, Tetradecanoic acid-5.46%, Catechin-23.23%, Aspidoperidin-17-ol-0,47% & Dibutyl phthalate-2.67% for sample A, alpha-Muurolene-0.37%, beta-Bisabolene-10.87%, E,Z-alpha-Farnesene-2.97%, 2,4-Di-tert-butylphenol-1.45%, Oleic acid-0.94%, (+)-epi-Biacyclosesquiphellandrene-2.07% & Apiole-0.16% for sample B, Tetradecane-1.24%, Octadecane-1.23%, 9-Eicosene-0.60%, Tetradecanoic acid-1.24%, Oleic acid-0.74%, Cis-9-Octadecenoic acid,hydroxyl-0.45%, 2,4-Di-tert-butylphenol-7.74%, 1,2-Diacyl-sn-glycero-3-phosphocholine-0.86%, alpha-Muurolene-0.37% & Dibutyl phthalate-0.88% for sample C and Octadecane-0.83%, 9-Eicosene-0.60%, alpa-Muurolene-0.47%, Dodecanoic acid-0.86%, Oleic acid-1.34%, 3-Chloropropionic acid-0.72%, Cis-9-Octadecenoic acid, 2-hydroxy ethyl-0.64%, 1,2-Diacyl-sn-glycero-3-phosphocholine-0.58% Catechin-18.54% for sample D. The bread samples have low moisture, moderate fat, protein, fibre content and high carbohydrate content making them nutritionally balanced. The presence of bioactive compounds like Catechin, alpha-Muurolene and beta-Bisabolene possessing antioxidant, anti-inflammatory and antimicrobial properties with several health benefits makes the bread samples of good nutritional quality, while the detection of Dibutyl phthalate, a contaminant linked to packaging materials though little, raises health concern and monitoring by relevant regulatory agencies is needed.

Keywords: *Proximate Analysis, Bread, Calabar, Chemical Compounds, GC-MS.*

I. INTRODUCTION

Bread is a staple food globally, and its composition varies according to ingredients and processing methods (Kumar et al., 2015). It consist of four main ingredients namely; flour, water, yeast and salt which comes together to form a dough that is then baked to create a fluffy and flavourful loaf of bread (Hamelman, 2004). In addition to the basic ingredients, other optional ingredients can be added to the bread dough to enhance its flavor, texture and appearance (Hensperger, 2010). These optional ingredients can include sugar, fat (like butter or oil), milk, or dairy products, eggs, herbs, spices, nuts, seeds and dried fruits. Sugar provides food for the yeast and helps to brown the crust during baking. Fats add richness and tenderness to the bread, while flavours like herbs, species, nuts, and seeds can be added to create unique and delicious bread varieties (Hensperger, 2010). Proximate analysis is a crucial tool for assessing the nutritional content or value of food products generally and bread in particular. According to AOAC (2015) and Oladeinde et al. (2015), proximate analysis is a standardized method for determining the nutrient content of food samples. It provides valuable insights into its macronutrients composition, including moisture, protein, fat, carbohydrates and ash content. Thus, it is a suitable method for evaluating the nutritional value and quality of bread. Quality control in bread production is very important and necessary to ensure its safety for consumption, and to safeguard the health of the people because it is the most readily available food in every home in Nigeria used to quench hunger and is relatively cheap, considered to have a balanced diet especially when taken with water or beverages. Above all, it is loved and eaten by all age groups, children and adults alike. As a result of this, the daily and annual consumption of bread is very high. The nutritional content of bread has a significant impact on public health, particularly in relation to micronutrient deficiencies and chronic diseases (WHO, 2018). The nutritional value and quality of bread depends on the ingredients or additives added to it during the baking or production process. This varies with the type or source of flour, leavening agents, sweetening agents, preservatives, flavouring agents, etc. Some of these additives are good for the body and some are not good due to their chemical nature or source. Leavening agents like natural yeast reacts with the lactic acid in milk to produce carbon (iv) oxide, a harmless gas to life which enlarges the air bubbles in the dough to rise during baking (Adow et al., 1991). Baking powder (Na_2CO_3) commonly called baking soda is often used in place of yeast for quick bread baking because it react to produce carbon (iv) oxide that rises the dough faster. Thus, the introduction/substitution of some natural ingredients with synthetic ones may sometimes alter the chemical composition or nutritional value of bread. However, there are concerns about the quality of flour and the presence of additives in commercially produced bread. Studies have revealed variations in nutritional content, with some breads being high in sugar and fats. According to Sivam et al.(2010), American bread types which includes white, whole wheat and specialty breads such as sourdough and multigrain, often contains added sugars and fats, which can impact health outcomes. White bread is reported to have a high glycemic index (GI), which can lead to rapid increases in blood glucose levels and is linked to insulin resistance and an increased risk of type 2 diabetes (Brand-Miller et al., 2003). Also, bread made with high levels of butter or margarine can contain unhealthy saturated fats that are linked to heart disease (Mozaffarian et al., 2010). Many commercially produced breads are enriched with vitamins and minerals but may also contain high sodium content (Baker, 2022). Excessive sodium intake is associated with hypertension and cardiovascular diseases (MacGregor et al., 2009).

German bread is known for its diversity including rye, spelt and multigrain varieties. It often incorporates whole grains and sourdough, which contribute to higher fibre content and a lower glycemic index (Miller, 2019). Whole grain breads are rich in dietary fibre which aids in digestion and reduces the risk of heart disease and type 2 diabetes (Slavin, 2005). French bread is typically simple with ingredients like flour, water, yeast and salt, often excludes fats and sugars. The artisanal methods used in baking French bread like fermentation periods, enhance its flavor and texture while contributing to lower glycemic responses (Leclerc, 2018). The effect of different types of flour on the chemical composition of bread was investigated by Aguilera et al., 2018) and reported that bread made from whole wheat flour had higher levels of fibre, protein and minerals compared to bread made from refined white flour. Adejuyitan et al. (2013) examined the use of composite flours from plantain and soybeans and revealed improvements in protein and fibre content while maintaining favourable sensory characteristics like taste and texture, as well as helping to fill the needed daily protein intake. Akubor et al. (2003) studied the partial replacement of wheat with cassava flour in bread making and found that the cassava-wheat bread had lower protein content and higher carbohydrate content. Also, the incorporation of sweet potato flour into wheat bread to boost beta-carotene content helps in enhancing its nutritional benefits without compromising its sensory quality (Akubor et al., 2003). According to Shrivastava and Chakraborty (2018), chickpea flour possesses amino acids that wheat flour lacks, like leucine, so mixing the two flours in some proportion supplement the protein content of wheat flour in bread formulations. The quality of bakery products is determined by variables like starch, water, protein and heat through baking traditionally (Srivastava et al., 2015). This implies that bread made from composite flour seems to balance the diet and possess several nutrients of good health benefits.

This study is aimed at assessing the nutritional quality of bread produced and consumed in Calabar, Nigeria with the view to ascertain its composition and the nutritional effects or benefits of consuming it. Besides, very little information is reported about bread in Calabar in literature in this perspective and the little available is about ten years ago, requiring regular monitoring and evaluation of bread quality in order to safeguard the health of the people since it is a staple food for people across all ages.

II. MATERIALS AND METHODS

- *Reagents:*

All reagents or chemicals used in this study were of analytical grade produced by the British Drug House, Poole, England. They include: Potassium iodide, starch indicator, copper (II) tetraoxosulphate acid. Concentrated Tetraoxosulphate (vi) acid, sodium hydroxide, methanol, distilled water, perchloric acid, Nitric acid, Acetic acid, ethanol, dilute ammonium hydrochloric acid.

- *Instruments and Apparatus:*

All instruments/equipment used in this study were in good condition and were calibrated accordingly following standard procedures. They include: Rotatory evaporator, Gas Chromatograph-Mass Spectrometer (BUCK M910

GC-MS equipped with HP-5MS column), Muffle furnace, weighing balance (Model GS-K load cell balance), water bath, hot plate, heating mantle, oven beakers, measuring cylinder, spatula, funnel, dessicator, filter papers, test tube, crucible Soxhlet extractor set, anti-bombing granules, round bottom flask. Litmus paper, conical flasks, Kjeldahl flask, tongs, volumetric flask, distillation sets, burette, pipette, indicator dropper, retort stand, electric stove stirrer.

- *Sample Collection and Preparation*

Four different most popular bread brands (samples) produced from Calabar and readily available for consumption were purchased from the bakeries within 2 to 3 days of production (baking). They were labelled A, B, C and D, and taken to the University of Calabar Chemistry Laboratory for further analysis. Each sample was bought consecutively and analyzed within 1 week, only GC-MS analysis was done for all the samples within 4 weeks from the beginning of analysis. This was done between August and September, 2025. Samples were preserved in a refrigerator when not in use, though for a short period. Each sample was homogenized by grinding before sub-divided into portions for each test or analysis.

- *Proximate Analysis*

The four bread samples were bought from four different bakeries and were analyzed according to the procedures reported as follows:

- *Moisture Content Determination*

The powdered samples (5 g) were weighed into pre-weighed crucibles and kept in an oven at 105^{0}C for 8 hours to dry to constant weight. The three crucibles and their contents were cooled in a desiccator and re-weighted. This process was repeated at 2 hours' interval for each of the samples.

- *Calculation*

$$\%\, Moisture\ content = \frac{(\text{Weight of sample before drying} - \text{Weight of sample after drying})\ \text{X}\ 100}{\text{Weight of sample taken}}$$

- *Determination of Crude Fat content*

- *Procedure:*

4 g of each of the powdered bread samples were weighed on a filter paper and transferred to the timble of the soxhlet extractor fitted on a pre-weighed 250 mL round bottom flask containing 150 mL petroleum ether and anti-bombing granules. The flask was connected to a reflux condenser and placed on an electric heater at a temperature of 60^{0}C to reflux for 45 minutes. After this period, the timble was removed from the soxhlet with the defatted sample. The petroleum ether was evaporated off from the flask using a rotatory evaporator. The lipid extract in the flask was then cooled in a desiccator and its weight measured.

Calculation: $\%\, Fat = \frac{\text{Weight of fat extract X 100}}{\text{Weight of Sample}}$

- *Determination of Ash content*

- *Procedure:*

2 g of each of the powdered bread samples was weighed into pre-weighed labeled crucibles and placed in the muffle furnace initially at low temperature of 28^{0}C, the temperature increased to 550^{0}C to ash the fruit, seed and leaf samples at 50 minutes, 40 minutes and 40 minutes respectively. The furnace was allowed to cool before removing the crucible with its content, the crucible was later cooled in a desiccator and re-weighed to get the ash content.

$$\%\, Ash = \frac{\text{Weight of ash x 100}}{\text{Weight of Sample}}$$

- *Determination of Crude Fibre Content Procedure*

The defatted bread sample (3 g) was weighed and transferred into a 400 ml beakers. 50 mL of 1.25% H_2SO_4 was added make up to 200 mL with distilled water and was boiled for 30 minutes, it was then cooled in a desiccator and filtered thereafter with a filter paper in a funnel. The residues were washed with hot water and tested for acid with litmus paper, this was done until the residues were acid free. The residues were transferred into the 400 mL beakers again and digested with 50 mL of 1.25% NaOH, made up to 200 mL with distilled water and boiled for another 30 minutes. The digests were allowed to cool, filtered and washed with hot water until the residues was alkaline free. The weight of the oven dried residues were noted after cooling in a desiccator.

- *Calculation:*

$$\%\, Crude\ fibre = \frac{\text{Weight of ash X 100}}{\text{Weight of defatted sample}}$$

- *Determination of Protein Content (Modified Kjeldahl Method)*

- *Procedure:*

The analysis was carried out in three stages, these were: The digestion stage, distillation stage and the titration stage.

- *Digestion Stage:*

The powdered bread samples (5 g) were weighed into 3 different 250 mL Kjeldahl flask. 0.5 g of each of the Kjeldahl catalysts (copper sulphate and sodium sulphate) were weighed into the Kjeldahl flasks. Anti-bombing granule was added and 50 mL of concentrated sulphuric acid was also added to the flask. The digestion flask was then place on the heating mantle to heat gently for an hour before being transferred to electric stove. The digestion process proceed with occasional swirling until a clear solution was obtained. The clear solution was transferred into a 100 mL standard flask and made up to the mark with distilled water.

- *Distillation Stage:*

10 mL of the digest was measured into the micro distillation apparatus; 12.5 mL of sodium hydroxide was also added to the flask. A condenser was connected from the distillation apparatus to a volumetric flask containing 5 mL of 5% boric acid and 2 drops of double indicator (methyl red and methyl blue). This was distilled for 50 minutes; the distillate was then titrated with 0.1M standard hydrochloric acid until a pale pink colour end point was obtained. The volume of acid used was noted. The blank determination was also carried out.

$$\%\,Nitrogen = \frac{\text{mL of HCl(sample)} - mL\ of\ HCl\ (blank) X\ molarity X\ 14 X\ 100\ X 100}{\text{mL of digest X Weight of sample X 1000}}$$

$$\%\,Crude\ protein = \%\,Nitrogen\ X\ Protein\ factor$$

Where the Protein factor and molar mass of Nitrogen are 6.25 and 14 respectively.

- *Determination of Carbohydrate Content*

The carbohydrate content of each sample using the formula below:

$$\%\,Carbohydrate = 100 - (\%\,Ash + \%\,Protein + \%Fat + \%Fibre)$$

- *Gas Chromatography-Mass Spectrometry (GC-MS)*

Fifty grams (50 g) of each sliced, dried and crushed powdered bread sample was weighed using an electrical weighing balance (Model GS-K load cell balance). Each weighed sample was wrapped in ash-less filter paper and put in the thimble of a Soxhlet extractor connected to a condenser and fitted to a distilling flask containing 250 mL of 99% ethanol. The sample was then extracted while heating on a mantle. Cold water was constantly supplied to the condenser through the water in-let to cool the system for good condensation of the solvent vapour. After Soxhlet extraction of the sample was done for about 3 hours, the solvent (ethanol) was evaporated using a rotatory evaporator and the extract was taken in a capped plastic container for GC-MS analysis.

For GC-MS analysis, it was performed on the BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length x 250 µm in diameter x 0.5 µm in thickness of film). Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1mL/min. The initial temperature was set at 50^{o}C with increasing rate of 3^{o}C/min and holding time of about10 min. Finally, the temperature was increase to 300^{o}C at 10^{o}C/min. One microliter of the prepared 1% of sample was diluted with acetonitrile and was injected in a split less mode. The observed components were compared with standards in the National Institute of Standards and Technology (NIST) library. Relative quantity of the chemical compounds present in each sample was expressed as percentage based on the peak area produced in the chromatogram.

III. RESULTS AND DISCUSSION

- *Results*:

The results of the analysis are presented in the Tables below thus; Table 1 shows the proximate analysis result, while Table 2A to 2D shows the GC-MS result of some chemical compounds present in the bread sample.

Table 1 Proximate Composition of the Four Bread Samples (% Dry Weight)

Sample	**Ash (%)**	**Protein (%)**	**Fat (%)**	**Fibre (%)**	**Moisture (%)**	**Carbohydrate (%)**
A	7.54±0.36	6.21±0.09	3.16±0.05.	3.34±0.40	4.46±0.78	78.29±0.51
B	3.53±0.24	3.40±0.25	1.63±0.35	0.35±0.16	5.81±0.54	85.29±0.70
C	8.89±0.30	4.26±0.10	2.61±0.48	0.43±0.08	6.37±0.29	77..44±0.40
D	2.78±0.67	8.46±0.28	4.17±0.53	0.58±0.21	6.39±0.35	77.62±1.31

N/B: Values reported in mean ± SD format, with N=3.

Table 1 above shows the results of the proximate composition of the four sample of bread and their mean values range between (2.78 – 8.89)% Ash, (3.40 - 8.46)% Protein, (1.63 - 4.17)% Fat, (0.35 - 3.34)% Fibre, (4.46 – 6.39)% Moisture and (77.44 – 85.29)% Carbohydrate.

Table 2A Some Chemical Compounds Present in Bread Sample A in Mg/L from GC-MS Analysis

Compound	Chemical formula	Retention Time (min.)	Area % Concentration
Hexadecane	$C_{16}H_{34}$	10.185	1.13
Heptadecane	$C_{17}H_{36}$	11.706	0.56
Octadecane	$C_{18}H_{38}$	11.165	2.17
Nonadecane	$C_{19}H_{40}$	14.559	0.72
9-Eicosene	$C_{20}H_{40}$	13.017	2.20
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	8.481	0.30
Dodecanoic acid	$C_{12}H_{34}O_2$	9.592	1.80
Tetradecanoic acid	$C_{14}H_{28}O_2$	12.556	5.46
Dibutyl phthalate	$C_{16}H_{22}O_4$	14.707	2.67
3-Chloropropionic acid	$C_3H_5ClO_2$	10.022	0.45
1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	13.331	0.40
Aspidosperidin-17-ol	$C_{21}H_{30}N_2O$	15.106	0.47
Catechin	$C_{15}H_{14}O_6$	15.320	23.23

Table 2B Some Chemical Compounds Present in Bread Sample B in Mg/L from GC-MS Analysis

Compound	Chemical Formula	Retention Time (min.)	Area % Concentration
alpha-Muurolene	$C_{15}H_{24}$	20.481	0.37
Beta-Bisabolene	$C_{15}H_{24}$	20.718	10.87
(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$	20.114	2.07
(E,Z)-alpha-Farnesene	$C_{15}H_{24}$	20.349	2.97
8-Isopropenyl-1,5-dimethyl-cyclo-1,5-diene	$C_{15}H_{24}$	20.588	0.37
3,4-Dimethylcyclohex-1-ene-4-carboxaldehyde	$C_9H_{14}O$	23.198	0.11
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	20.982	1.45
tau-Muurolol	$C_{15}H_{26}O$	23.983	0.21
Oleic acid	$C_{18}H_{34}O_2$	31.994	0.94
Apiole	$C_{12}H_{14}O_4$	23.598	0.16

Table 2C Some Chemical Compounds Present in Bread Sample C in Mg/L from GC-MS Analysis

Compound	Chemical Formula	Retention Time (min.)	Area % Concentration
Tetradecane	$C_{14}H_{30}$	18.476	1.24
Octadecane	$C_{18}H_{38}$	6.884	1.23
alpha-Muurolene	$C_{15}H_{24}$	20.481	0.37
9-Eicosene	$C_{20}H_{40}$	37.258	0.60
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	22.133	7.74
Oleic acid	$C_{18}H_{34}O_2$	32.993	0.74
1,2-Diacyl-sn-glycero-3-phosphocholine	$C_{18}H_{32}O_2$	32.601	0.86
2-Hydroxypropanoic acid	$C_3H_6O_3$	30.887	0.62
Cis-9-Octadecenoic acid, 2-hydroxyethyl	$C_{18}H_{34}O_3$	5.253	0.45
Dibutyl phthalate	$C_{16}H_{22}O_4$	30.527	0.88

Table 2D Some Chemical Compounds Present in Bread Sample D in Mg/L from GC-MS Analysis

Compound	Chemical Formula	Retention Time (min.)	Area % Concentration
Tetradecane	$C_{14}H_{30}$	17.976	0.91
Octadecane	$C_{18}H_{38}$	7.104	0.83
alpha-Muurolene	$C_{15}H_{24}$	20.201	0.47
9-Eicosene	$C_{20}H_{40}$	37.258	0.60
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	21.523	5.64
Oleic acid	$C_{18}H_{34}O_2$	31.763	1.24
3-Chloropropionic acid	$C_3H_5ClO_2$	9.461	0.72
Dodecanoic acid	$C_{12}H_{34}O_2$	8.362	0.86
Cis-9-Octadecenoic acid, 2-hydroxyethyl	$C_{18}H_{34}O_3$	4.683	0.64
1,2-Diacyl-sn-glycero-3-phosphocholine	$C_{18}H_{32}O_2$	30.217	0.58
Catechin	$C_{15}H_{14}O_6$	14.850	18.54

- *Discussion*

- *Ash Content*

The mean ash content of the four bread sample as shown in Table 1 were 7.54, 3.53, 8.89 and 2.78% for A, B, C and D respectively. Bread sample C had the highest ash content 8.89, while sample D had the lowest value of 2.78.Ash content indicates level of mineral content of a material. Lower ash content of food sample also indicate higher quality for energy production and vice versa (Olubunmi et al., 2019).The ash content range (2.78 – 8.89%) of this study agrees with those reported by (Olubunmi et al., 2019) in their study on bread which has a value range of (2.20 – 9.01%).

- *Moisture Content*

The mean moisture content of bread samples were determined to be 4.46, 5.81, 6.37 and 6.39% for A, B, C and D respectively as shown in Table 1. Bread sample D had the highest moisture content of 6.39%, while sample A had the lowest value of 4.46%. Low moisture content of bread indicates that it can be stored for longer period of time (prolonged shelf-life) and also higher resistance to microbial action and contamination. The moisture content range (4.46 – 6.39%) of bread samples of this study are lower than (11.90 – 13.23%) reported by (Olubunmi et al., 2019) in a similar study elsewhere. This reveals that bread consumed in Calabar have longer shelf-live and higher resistance to microbial action.

- *Fibre Content*

The fibre content represents the indigestible or rough matter in food (Sathe, 1999). From the results (Table 1), the mean values of the four bread sample were 3.34, 0.35, 0.43 and 0.58% for A, B, C and D respectively. Bread sample A had the highest fibre content of 3.34%, while sample B had the lowest value of 0.35%. The fibre content of food also indicates the presence of cellulose, hemicellulose and lignin. It provides insight into the nutritional value, digestibility, textural properties and energy density of food (Van et al., 2005). Higher fibre content of food is beneficial for health, aiding digestion, and help manage chronic diseases. Bread samples of this study had lower fibre content (0.35 – 3.34%) compared to (1.07 – 3.50%) reported by (Olubunmi et al., 2019) in a similar study.

- *Carbohydrate Content*

The mean carbohydrate content of the four bread samples as shown in Table 1 are 78.29, 85.29, 77.44 and 77.62% for A, B, C and D respectively. Bread sample B had the highest carbohydrate content of 85.29%, while sample C had the lowest value of 77.44%. Carbohydrates plays a paramount role as a source of energy both in human food and animal feed. The results of this study shows high carbohydrate contents in all the bread samples indicating that carbohydrates as the major nutrient in bread products. Olubunmi et al. (2019) in a similar study reported a carbohydrate content range of (60.01 – 73.67%) slightly lower than (77.44 – 85.29%) determined in this study, confirming that bread is mainly a carbohydrate food product.

- *Crude fat Content*

The mean crude fat content of four bread samples as determined in (Table 1) are 3.16, 1.63, 2.61 and 4.17% for sample A, B, C and C respectively. Sample D had the highest crude fat content of4.17%, while sample B had the lowest value of 1.63%. Fat content of a food sample is vital because it determines the palatability of the product and also prolongs its shelf-life. It is also a criteria for assessing nutritional, industrial and economic value of the food product. The low fat content (1.63 – 4.17%) of bread samples in this study, though higher than (0.77 – 1.41%) reported by (Olubunmi et al., 2019) elsewhere indicates that the bread samples are healthy but may lack some sensory and textural appeal, as high fat content in food sample tend to be richer and more indulgent. However, as low fat content of bread affects the textural appeal, it is recommended for obese individuals (Olubunmi et al., 2019).

- *Protein Content*

The result of the analysis (Table 1) revealed the mean crude protein content of the bread samples to be 6.21, 3.40, 4.26 and 8.46% for A, B, C and D respectively. Bread sample D had the highest protein content of 8.46%, while sample B had the lowest value of 3.40%. Proteins help in building and repairing worn out tissues, and is also used to assess nutritional value, quality and economic importance of food. High protein content is desirable in both human and animal diets as it supports growth, repair tissues and enhance metabolic functions. The protein content value range (3.40 – 8.46%) of bread samples in this study are lower than (11.37 – 16.77%) reported by (Olubunmi et al., 2019) in a similar study elsewhere. This variation could be as a result of different additional ingredients that were added other than the common bread condiments.

The results form GC-MS analysis for bread sample A in Table 2A revealed the presence of many compounds, some of which were saturated and unsaturated hydrocarbons, fatty acids, alcohol, esters and other aromatic/organic compounds. One of the notable compounds found is catechin ($C_{15}H_{14}O_6$), a flavonoid with antioxidant properties, contributing a significant part of the bread's composition of 23.23%. The presence of catechin indicates a potential source of health benefits due to its role in neutralizing free radicals in the body. Also, compounds like 9-Eicosene ($C_{20}H_{40}$), Octadecane ($C_{18}H_{38}$) and Nonadecane ($C_{19}H_{40}$) suggest the presence of long-chain hydrocarbons that may be associated with the oils and fats used in bread making. Saturated hydrocarbons are typical in bread, likely contributing to the texture and moisture retention in the product. Dodecanoic acid ($C_{12}H_{24}O_2$) a saturated fatty acid, also called lauric acid was detected at 1.80% and is commonly found in coconut oil, palm kernel oil and other sources. Its presence is vital as it has antimicrobial properties and contributes to the bread's stability and shelf-life. The detection of Dibutyl phthalate ($C_{16}H_{22}O_4$), a phthalate ester usually used as a plasticizer, though low at 2.67% is of concerned. It may indicate contamination from the packaging materials for the bread or raw materials or industrial sources because it is a known environmental contaminant with potential health risk. Its presence is of concerned from food safety of the bread perspective no matter how little.

The results in Table 2B revealed that beta-Bisabolene ($C_{15}H_{24}$), a sesquiterpene with antimicrobial and anti-inflammatory properties is the most abundant compound at 10.87% concentration. It may contribute to the flavor and the preservation The presence of (E,Z)-alpha-Farnesene. ($C_{15}H_{24}$) and (+)-epi-Bicyclosesquiphellandrene ($C_{15}H_{24}$) further indicates the essential oils or flavor additives used in bread making. Terpenes are common in aromatic plants and may give distinct flavor characteristics. Interestingly, bread sample B also contains 1.45% concentration of 2, 4-Di-tert-butylphenol ($C_{14}H_{22}O$), an antioxidant commonly used to prevent oxidation in food products. This indicates that it source could be from the additives during the formulation process to prolong the shelf-life and stability of the bread. Apiole ($C_{12}H_{14}O_4$) was also detected in sample B at 0.16% indicating the use of vegetable oil of celery leaf or parsley seed and it can act as a lactating agent in nursing mothers among other uses.

The results in Table 2C shows the presence of hydrocarbons, fatty acids and additives like 2,4-Di-tert-butylphenol ($C_{14}H_{22}O$) at 7.74% concentration, revealing the presence of both natural and synthetic additives that contributes to the bread's quality, flavor and shelf-life. The detection of Cis-9-octadecenoic acid, 2-hydroxyethyl ($C_{20}H_{38}O_3$), a fatty acid derivative indicates the use of plant-based oils or emulsifiers in the bread production as it is commonly found in margarine and other processed fats, potentially contributing to the bread's texture. Similar to the findings in sample A, is the identification of Dibutyl phthalate ($C_{20}H_{38}O_3$) in sample C at 0.88% concentration, which raises food safety concern and reinforces the need to investigate the sources of contamination from packaging materials. 1,2-Diiacyl-sn-glycero-3-phosphpcholine ($C_{18}H_{34}O_8P$), a phospholipid was also present in the bread sample indicating the use of natural and synthetic emulsifiers in the production process of the bread to maintain its structure and texture. Phospholipids are common in processed foods, giving moisture retention and improving the product's consistency.

The results in Table 2D for bread sample D revealed the identification of compounds like 9-Eicosene ($C_{20}H_{40}$), Octadecane ($C_{18}H_{38}$) and Tetradecane ($C_{14}H_{30}$) suggesting the presence of long-chain hydrocarbons that may be associated with the oils and fats used in bread making, and similar to sample A. Saturated hydrocarbons are normally present in bread, as the likely contribute to the texture and moisture retention in the product. The presence of saturated and unsaturated fatty acids namely; Dodecanoic acid ($C_{12}H_{24}O_2$), Oleic acid ($C_{18}H_{34}O_2$), 3-Chloropropionic acid ($C_3H_5ClO_2$) and Cis-9-octadecenoic acid, 2-hydroxyethyl ($C_{20}H_{38}O_3$), at 0.86, 1.24, 0.72 and 0.64% concentration respectively indicates the use of plant based oils or emulsifiers like in the case of sample B. Catechin ($C_{15}H_{14}O_6$), a flavonoid with antioxidant properties and beneficial to human health as it can neutralize free radicals in the body was also detected in sample D at 18.54% concentration slightly lower than sample A at 23.23%. Also, 2,4-Di-tert-butylphenol, an antioxidant and 1,2-Diiacyl-sn-glycero-3-phosphpcholine, a phospholipid was detected in bread sample D like sample C. The presence of alpha-Muurolene ($C_{15}H_{24}$), a sesquiterpene and a carbobicyclic compound indicates the use of plant-based terpenes notable for their antimicrobial and antioxidant properties that are beneficial to the human body and also prevent the fast oxidation of the bread sample, thereby preserving and prolong the shelf-life of the bread.

The presence of long chain hydrocarbons in the bread samples especially A, C and D like pentadecane and hexadecane in bread, and these hydrocarbons are often associated with the use of vegetable oils and fat substitutes in bread formulations. In addition, the presence of fatty acids in the bread samples could also be due to the use of vegetable oils and fats like margarine or butter in bread production. The composition of these fatty acids varies depending on the type of bread as there may be lower concentrations of fatty acids in gluten-free bread compared to the traditional wheat bread samples in this study. Antioxidants like 2, 4-Di-tert-butylphenol found in all the bread samples as shown in the Tables above are used to improve the stability/shelf-life of the thread and preserve it. According to Czajkowska-Gonzalez et al. (2021), bread produced using white wheat flour obtained after removing the wheat bran and germ where fibre, phytochemicals and important essential nutrients are normally found. Consequently, the final product (wheat flour) contains low dietary fibre and phenolic compounds (phytochemicals) (Xu et al., 2019) To make up for this loss of nutrients and other bioactive compounds, foods enriched with natural antioxidant like phenolic compounds (Czajkowska-Gonzalez et al. 2021) and even synthetic antioxidants are sometimes added to improve the health properties of bread. Examples of such synthetic antioxidants are Butylated hydroxytoluene (BHT and Butylated hydroxyanisole (BHA) among others. However, BHT and BHA were not found in the bread samples in this study. Thus, the consistent use of antioxidants in bread across different regions indicates that preserving the freshness of bread is a necessity and a universal challenge in the industry. It has also been reported that the antioxidant potential in bakery products strongly depends on the manufacturing conditions (Han and Koh, 2011; Dziki et al., 2014). Antioxidant activity and free phenolic acid levels were reduced by mixing of ingredients but were recovered by fermentation and baking (Dziki et al., 2014). They explained that bonds with antioxidants are hydrolysed during fermentation, liberating antioxidants.

Bread rich in bioactive compounds with antioxidant properties is of high quality because it is nutritious and healthy. Antioxidant compouds like phenolic acids, polyphenols and flavonoids can trap free radicals such as peroxide, hydro-peroxide or lipid peroxyl and thus hinder the oxidative mechanisms that causes degenerative diseases (Emad, 2014).

The presence of Catechin, a bioactive flavonoid and a well-known antioxidant with several health benefits in the bread samples in this study at a high level especially samples A and D is interesting and beneficial to the consumers. It also makes the findings of this study unique as catechin has not been widely reported in previous studies as one of the bioactive compunds in bread compared to other bioactive compounds with antioxidant properties like ferulic acid and coumaric acid. This suggest that bread in Calabar may contain ingredients like certain whole grains or additives that are not commonly used in other regions, leading to the high levels of catechin observed in this study. One of the major advatges of the presence ocatechin in wheat flour or bread is the fact that catechins retain strong antioxidant activity even after baking as reported by Pan et al. (2022) in their study. Besides, the presence of sesquiterpernes like alpha-Muurolene and beta-Bisabolene possessing antioxidant, anti-inflammatory and antimicrobial properties in the bread samples especially B and D is an indication that some bread samples in Calabar possess nutritional and health benefits.

The detection of Dibutyl phthalate (DBP) in samples A and C (2.67% and 0.88% respectively) indicates potential contamination from packaging materials of plastics origin, used to package either the flour or the bread product itself. Phthalate esters as plasticizers are not physically bound to polymeric materials and as such, they are easily lost from the materials into food products (Shi et al., 2016; Vavrous et al., 2016). Dibutyl phthalate (DBP) is one of the most frequently detected phthalate esters in in Chinese dietary foods though lower in quantity compared to other countries (Yang et al., 2018). Since bread is often packaged or wrapped in polythene or plastics related materials, the tendency for DBP to be transfer into the bread after sometime is possible, especially in the tropics like Nigeria where room temperatures are sometimes above 30^{o}C due to heat. Though very little, the detection of DBP in some bread samples raises concern in safeguarding the health of the people and should be monitored by relevant regulatory agencies.

IV. CONCLUSION

The study revealed from the proximate analysis that the bread samples have low moisture, moderate fat, protein, fibre content and high carbohydrate content making them nutritionally balanced. The GC-MS analysis revealed the presence of antioxidants making the bread stable with long shelf-life coupled with their low moisture content reported. The bread also contain long chain hydrocarbons and fatty acids due to the use of vegetable oils and fats substitutes in their production as emulsifiers. Above all, the presence Catechin, a bioactive flavonoid with several health benefits and sesquiterpernes like alpha-Muurolene and beta-Bisabolene possessing antioxidant, anti-inflammatory and antimicrobial properties makes the bread samples of good nutritional quality and beneficial health wise. However, the detection of Dibutyl phthalate, a plasticizer and contaminant linked to packaging materials though little, raises concern in safeguarding the health of the people and should be looked into by relevant regulatory agencies.

- *Conflict of Interest:*

  The authors declare that there is no conflict of interest with the manuscript.

- *Ethical Approval:*

  Nil, Animals were not involved and Human Rights not violated.

- *Funding:*

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