
ASSESSMENT OF ANTIOXIDANT LEVELS AND MICROBIOLOGICAL QUALITY OF SELECTED BRANDS OF BISCUITS SOLD IN BENUE STATE, NIGERIA.

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Abstract

Biscuit has been the most popular bakery product consumed worldwide and it is acceptable in all age group. They are usually made from wheat flour together with fat, milk, sugar and salts. Food additives such as Butylated hydroxytoluene, sodium metabisulphite and citric acids are often added to the traditional ingredients to increase its shelf-life and improve its organoleptic properties and some additives are known to be carcinogens. The need therefore to conduct an empirical assessment of some food additives level and microbiological quality of some common biscuit brands sold in Benue State, Nigeria. Ten brands were used for this study. Five packs of each brand were composited to obtain two composite samples per brand moisture content, Standard methods using HLCP were used to determine the concentration of Butylated hydroxytoluene (BHT) and Sodium Metabisulphite (SMS) while titration method was used for Citric Acid (CA) concentration. Pour plate technique was used to determine total aerobic plate count and colony morphology and biochemical characteristics was used to identify the most probable microbial contaminants of the biscuits, the moisture contents were of standard values, the mean aerobic count of the biscuit samples ranged from 1.1×10^1 to 2.4×10^1 cfu/g, the total mold/fungi count ranged from 1.1×10^1 to 1.2×10^1 cfu/g. The following bacteria and fungi were found to be common contaminants of biscuits sold in Benue State; Staphylococcus epidermidis, Bacillus subtilis, Aspergillus niger and Penicillium sp. All the antioxidants checked for were within the safety acceptable limits of WHO and were all coliform free, therefore are safe for consumption.

Keywords: Antioxidant level, Microbiological quality, Biscuits, Butylated hydroxytoluene, sodium metabisulphite, citric acids

Introduction

Biscuits are baked product, usually made from wheat flour together with fat, milk, sugar and salts (Mamat and Hill, 2018). They are made in a variety of shapes and sizes and may contain dry fruits, nuts and food colors. They are dry product, usually with a golden brown color and a crisp texture. The major raw ingredients used for the preparation of biscuits are 44% flour, 23% sugar and 3 % milk, 11% fat and 4% eggs giving an average chemical composition of 6% protein, 28% fats and 57% carbohydrate 1% fibres for an average energy contain of approximately 510 Kcal/100g (Pasqualone *et al.*, 2021). Due to the growing consumer demands for convenient food, biscuits represent one of the fast-growing segments of bakery products. Ready to eat, unique taste, readily available, long shelf life, Biscuits are ready to eat, convenient and cheap snacks that are consumed by all age group in many countries (Adebowale *et al.*, 2012). Food additives are substances that food manufacturers intentionally add to food in small quantities during production or processing to improve the organoleptic properties of the food. Butylated hydroxytoluene, sodium metabisulphite and citric acids are some of the most common food additives used in biscuits and other manufactured food products. The risk associated with the ever increasing use of food additives in food products poses a huge risk to human immune system leading to various disorders and diseases such as food allergies, food intolerance, cancer, multiple sclerosis (MS), attention deficit hyperactivity disorder (ADHD), brain damage, nausea, cardiac disease amongst others (Fernandes, Carvalho and Guido, 2019). Food additives must be used at a considerably safety limit to ensure safety for a lifetime of its consumption based on current toxicological evaluation.

Material and Method

A pilot survey on biscuits commonly sold in Benue State

This was conducted in main markets of nine (9) major towns in the three senatorial districts of Benue State that is Makurdi, Otukpo, Gboko, Katsina Ala, Otukpa, Aliade, Ogbia, Ugbokolo and Zakibiam. Brands and manufacturers of biscuits commonly available in the entire sample locations were determine and ten brands were found to be common in all the markets.

Sample Procurement and Preparation

Samples of the following biscuit brands Parle G, Coaster, McVite Digestive, Oxford Cabin, Festo, Lunch pack, Malt & Milk, Mcvite Rich tea, Yale Cabin and spicy were purchased from Otukpo Central market and Ugbokolo market in Benue State. Ten (10) samples from separate batches were procured for each brand of biscuit bringing the total number of samples collected to hundred (100) packs of biscuits. For each biscuit brand, five packs each from a given batch number were composited to obtain 2 composite samples per brand which were pulverized to give two representative samples labeled (A and B). Moisture content and microbiological qualities of the biscuits were determined immediately after pulverization of the composites. Each of the composite samples was stored in dark amber bottle until the determination of butylated hydroxytoluene (BHT), sodium metabisulphite (SMS) and citric acid in each of the sample.

Analysis of Colour

This was done following the method of Fernandes, Carvalho and Guido (2019). The biscuits' colours were analyzed with the Minolta CR-410 colorimeter. The parameters used were Luminosity (L), Red (a), and Yellow (b). The biscuits were analyzed in their form of

consumption (without being ground), so that the color could be considered a method of control in future industrial tests and quality parameters.

Moisture Content Determination

The moisture content was also determined following the method of Fernandes, Carvalho and Guido (2019). The biscuits' moisture level was assessed on the same day the samples were bought from the stores. About 5 g of ground biscuit were dried for 3 h at 100 °C. After drying and cooling, the dry mass of the biscuit was measured and the moisture content was calculated.

Total Aerobic bacteria and fungi plate count

The standard method described by Cheesbrough (2006) was adopted with little modifications, twenty (20) gram of each biscuit samples was homogenized in 200mL diluents (Sterile Physiological saline) this form the stalk and it was labeled as 10^{-1} from 1mL was transferred into another test tube containing 9mL of the diluents, labeled 10^{-2} this procedure was repeated until the seventh fold labeled 10^{-7} . After which 1mL from each of the dilutions was pipette into pre-labeled Petri dishes for total aerobic bacteria and fungi count as well as coli form bacteria count.

Autoclaved sterile nutrient agar that has been cool to about 35°C was poured into the inoculated plates labeled for total aerobic bacteria count, while McConkey agar was used for total coliform bacteria count, PDA was used for total fungi count. The plates were rocked gently on the work bench for homogeneity of agar and the inoculums and allowed to solidify, then incubated at 35°C, total viable aerobic bacterial count was recorded after 24hours of incubation while total fungi plate count was done after 72hours. Plates containing 25-250 colonies were counted and multiplied by the dilution factor to get the number of colony forming unit (CFU) per gram of the sample. Miles and Misra(1938) method was used for the enumeration. The inoculation was done in duplicate and the mean values of the plate count were used for the calculation. The total viable count was then obtained by using the formula as shown below.

$$\text{Total viable count (cfu/ml (colony forming unit per milliliter))} = \frac{N}{V} \times D$$

Where N = Mean colony count

V = volume plated

D = Dilution factor

Identification of Bacteria Isolated

The isolates were subculture by repeated streaking on their respective media until pure cultures were obtained. The pure culture of bacteria was subjected to Gram reaction, motility, catalase, oxidase, indo, sugar fermentation, methyl red and gas production tests. The most probable bacterial isolates were identified based on their cultural and microscopic morphology and the biochemical tests conducted as described by Bergey and Holt (2000).

Identification of Fungi

Identification of fungi was done according to the methods described by Muhamad *et al.* (2018). A drop of lactophenol cotton blue stain was placed on clean slide and with the aid of mounted needle; a small portion of the mycelium from the fungal cultures was removed and placed in the drop of the stain. The mycelium was spread very well on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The slide was then examined under the microscope. The observation was done at high power objective (X40) of the microscope. The cultural characteristics such as colony arrangement and pigmentation and microscopic morphology of hyphae type were observed and used for the fungi identification.

Determination of Butylated Hydroxytoluene (BHT)

This was determined using High Pressure Liquid Chromatography (HPLC) system under the following set of conditions;

- i. Column = C18 (250 x 4.6 mm) 5 µm particle size
- ii. Mobile phase= methanol: water: glacial acetic acid at (75%: 24%: 1%)
- iii. Wavelength = 285 µm
- iv. Injection volume = 20 µl
- v. Flow rate = 1 mL per minute
- vi. Column temperature = 28°C

Preparation of Standard Solution

Ten milligram of standard butylated hydroxytoluene (pellets) was weighed into a 25 mL flask, 10 mL of methanol was added and vortex for 5 minutes and the solution was made up to the mark with methanol to make the stock solution (0.5 mg mL⁻¹). Four millilitre of the stock solution was transferred into another 25 mL flask and the solution was made up to the mark with methanol to make the intermediate solution. One millilitre of intermediate solution was pipetted into a 10 mL volumetric flask and made up to the mark with methanol after which the concentrations were adjusted to prepare working solutions at 2, 3, 4 and 5 mL respectively. Each level of preparation was filtered using a 0.45 µm filter and the filtrate was transferred into auto sample vials and corked. Each of the calibrant was injected into the HPLC machine using auto sampler.

Sample Preparation

One gram of sample was weighed into a 10 mL volumetric flask and 1 mL of anhydrous ethanolic solution containing 0.25% glacial acetic acid was added to the sample. The sample was placed in an ultrasonic bath for 5 minutes and homogenized using a magnetic stirrer (750 rpm). The solution was centrifuged for 5 minutes at 3000 rpm and the ethanolic phase was collected. The extraction was repeated for two more times and thereafter the 3 extractions were combined and dried passing a N₂ gas stream over it. The dried residue will be reconstituted in 1 mL methanol and injected twice into the HPLC sample port.

Calculations were done using;

$$\text{Conc. (mg)} = \text{mg from calibration curve} \times \text{mass} \times \text{dilution factor} / \text{weight taken} \text{-----} (1)$$

Where mass = manufacturer recommended serving size

Determination of Sodium Metabisulphite (SMS)

This was determined using High Pressure Liquid Chromatography (HPLC) system under the following set of conditions;

- i. Column = C18 (250 x 4.6mm) 5 µm particle size
- ii. Mobile phase = 0.1% phosphoric acid: acetonitrile
(60 : 40)
- iii. Wavelength = 275 µm
- iv. Injection volume = 20µL
- v. Flow rate = 1.2 mL per minute
- vi. Column temperature = 28 °C

Preparation of Standard Solution

Ten milligram of standard sodium metabisulphite (pellets) was weighed into a 10 mL flask and 5 mL of diluents was added and vortex for 5 minutes, then the mark was made up with the diluents.

One mL of the stock solution was transferred into another 20 mL flask and made up to the mark with the diluents to make the intermediate solution. One milliliter of intermediate solution was pipette into a 10 mL volumetric flask and made up to the mark with the diluents after which the concentrations were adjusted to prepare working solutions at 2, 3, 4 and 5 mL respectively. Each level of preparation was filtered using a 0.45 µm filter and the filtrate was transferred into auto sample vials and corked. Each of the calibrant was injected into the HPLC machine using auto sampler.

Sample Preparation

Two gram of sample was weighed into a 25 mL volumetric flask and 15 mL of diluents was added to the sample. Then the sample was placed in an ultrasonic bath for 5 minutes, the mark was made up with diluents and it was filtered with Watman No. 1 filter paper. The filtrate was transferred into auto sample vials and corked using a 0.45 µm membrane and then injected into the HPLC machine.

Calculations were done using;

$$\text{Conc. (mg)} = \text{mg from calibration curve} \times \text{mass} \times \text{dilution factor} / \text{weight taken} \text{-----} (2)$$

Where mass = manufacturer recommended serving size

Determination of Citric Acid

Citric acid content was done following the methods of David, (2014). Ten grams of sample was accurately weighed and the weighed sample was dissolved with 30 mL of water in a beaker, then transferred into a 100mL volumetric flask and it was made up to the mark with water. The mixture was placed on a vortex mixer for 10 minutes until every particle was dissolved and the mixture was allowed to stand for 20 minutes. The solution was decanted and the clear part of the solution was taken. Ten milligrams of the clear solution was measured in duplicate, 2 to 3 drops of phenolphthalein indicator was added. The burette was filled with 0.1M sodium hydroxide (NaOH) and amount of sodium hydroxide required to neutralize the acid gives the acidity of the sample. The sample was titrated against 0.1M NaOH till a pink colour was observed.

The titre value was noted and the total acidity calculated using equation 3.

Statistical Analysis

Means and standard deviations of BHT, SMS and CA in each of the biscuit brands analyzed were calculated and compared by One-Way Analysis of Variance (ANOVA) using SPSS

version 22 statistical software package to determine if their levels in the different biscuit brands are significantly different at $p > 0.05$.

RESULTS AND DISCUSSION

The result of moisture content as well as colour of the selected biscuit is shown in table 1. The values of moisture content ranged from 0.5 to 1.2% and are all within the standard (0.1 - 5%) in biscuits. The colours are natural ranging from yellow to brown and looks appealing.

The results of antioxidants levels in selected biscuit brands sold in Benue State are shown in table 2. The result shows that the level of butylated hydroxytoluene (BHT) ranged between 0.000115 mg/g to 0.000295 mg/g. Festo biscuit had the highest BHT concentration while Lunch pack biscuit had the lowest concentration of BHT. Sodium metabisulphite (SMS) concentration in the biscuits sampled ranged between 0.0001115 mg/g to 0.001475 mg/g. Malt and Milk biscuit had the highest concentration of SMS while Oxford cabin biscuit had the lowest concentration of SMS. The Citric Acid concentration of the selected biscuit brands ranged from 0.019100 mg/g to 0.1326000%. Parle G biscuit had the highest concentration of citric acid while Yale Cabin had the lowest concentration of citric acid.

The result of aerobic microbial plate count is shown in table 2. The total aerobic bacterial plate count of the selected biscuit ranged between 1.1×10^{-1} to 2.4×10^{-1} cfu/g. Yale cabin biscuit had the highest aerobic bacterial count while McVites digestive biscuit had the lowest aerobic bacterial count. There was no growth of coliform bacteria in all the biscuit brands examined. The total fungal count of the selected brand of biscuit ranged between 1.1×10^{-1} to 3.2×10^{-1} cfu/g. Yale cabin had the highest fungal count while festo biscuit had the lowest fungal count.

Table 1: table showing the moisture content and colour of sampled biscuit

S/No	Biscuit brands	Moisture content (%)	Colour
1	McVitie Rich Tea	0.60	Caramel
2	McVites Digestive	0.80	Caramel
3	Malt and Milk	0,50	Light brown
4	s p i c y	0,70	Light yellow
5	F e s t o	0,60	Chocolate brown
6	L u n c h P a c k	0.80	Brown
7	C o a s t e r	1.00	Light brown
8	P a r l e G	0,80	Chocolate brown
9	O x f o r d C a b i n	1.20	Light brown
10	Y a l e C a b i n	1.00	Light brown

The Result of Biochemical Characterization of Bacterial Isolates from selected Biscuit brands sold in Benue State is shown in table 3, two bacterial species were found to be common contaminants of all the selected biscuit brands they are *Staphylococcus epidermidis* and *Bacillus subtilis*. Plate 1 shows the picture of *Aspergillus niger* isolated from the biscuit brands.

Table 2: Concentration of Antioxidants in Selected Biscuit Brands Sold in Benue

S/No	Brand of Biscuits	B H T (m g / g)	S M S m g / g)	C A (%)
1	McVitie Rich Tea	0.000235±0.000212 ^{c,d}	0.001455±0.000064 ^c	0.031760±0.000354 ^b
2	McVites Digestive	0.000250±0.000283 ^{c,d}	0.001275±0.000050 ^b	0.030755±0.000347 ^b
3	Malt and Milk	0.000230±0.000566 ^c	0.001475±0.000050 ^c	0.049565±0.002044 ^c
4	S p i c y	0.000135±0.000135 ^{a,b}	0.001280±0.000028 ^b	0.116550±0.006435 ^e
5	F e s t o	0.000295±0.000295 ^{d,e}	0.001510±0.000057 ^c	0.032201±0.001273 ^b
6	L u n c h P a c k	0.000115±0.000122 ^a	0.001295±0.000021 ^b	0.065615±0.000573 ^d
7	C o a s t e r	0.000285±0.000354 ^{d,e}	0.001290±0.000028 ^b	0.032560±0.000778 ^b
8	P a r l e G	0.000225±0.000071 ^c	0.001525±0.000021 ^c	0.132600±0.000707 ^f
9	Oxford Cabin	0.000195±0.000021 ^{b,c}	0.001115±0.000007 ^a	0.021505±0.000700 ^a
10	Yale Cabin	0.000220±0.000141 ^c	0.001130±0.000014 ^a	0.019100±0.001414 ^a
11	Maximum permitted limit in Food	0 . 0 7 - 0 . 2	0 . 0 1 5 - 0 . 5	0 . 5 - 2 . 0
12	Acceptable Daily Intake	0-0.0003mg/g body weight/day	0.0007mg/g body weight/day	0 . 5

BHT: Butylated hydroxytoluene

SMS: Sodium metabisulphite

CA: Citric Acid.

Table 3: Aerobic Microbial Plate Count of selected biscuit brands sold in Benue State.

S/No	Brand of Biscuits	Aerobic bacterial count (CFU/g)		Coliform bacterial count (CFU/g)		Total fungal count (CFU/g)	
1	McVitie Rich Tea	1 . 2	x 1 0 ⁻¹	0	-	0	2 . 1 x 1 0 ⁻¹
2	McVites Digestive	1 . 1	x 1 0 ⁻¹	0	.	0	2 . 1 x 1 0 ⁻¹
3	Malt and Milk	1 . 3	x 1 0 ⁻¹	0	.	0	1 . 3 x 1 0 ⁻¹
4	S p i c y	1 . 3	x 1 0 ⁻¹	0	.	0	2 . 2 x 1 0 ⁻¹
5	F e s t o	1 . 4	x 1 0 ⁻¹	0	.	0	1 . 1 x 1 0 ⁻¹
6	L u n c h P a c k	1 . 6	x 1 0 ⁻¹	0	.	0	2 . 3 x 1 0 ⁻¹
7	C o a s t e r	1 . 3	x 1 0 ⁻¹	0	.	0	2 . 5 x 1 0 ⁻¹
8	P a r l e G	1 . 3	x 1 0 ⁻¹	0	.	0	2 . 2 x 1 0 ⁻¹
9	Oxford Cabin	2 . 1	x 1 0 ⁻¹	0	.	0	3 . 1 x 1 0 ⁻¹
10	Yale Cabin	2 . 4	x 1 0 ⁻¹	0	.	0	3 . 2 x 1 0 ⁻¹

CFU/g = Colony forming Unit per gram

Table 4: Result of Biochemical Characterization of Bacterial Isolates from selected Biscuit brands sold in Benue State.

Isolates	Cell morphology	Cell motility	Gram Reaction	Catalase	Oxidase	Coagulase	Urase	Citrate	Indole	Hemolysis	Glucose	Fructose	Lactose	Sucrose	Methyl red	Gas production	Most probable organism
1	C	-	+	+	-	-	+	-	-	-	+	+	+	+	-	+	<i>Staphylococcus epidermidis</i>
2	R	+	+	+	+	-		+	-	+	+	+	+	+	-	-	<i>Bacillus subtilis</i>

C= Cocci

R= Rod

+ = positive test

- = Negative test

Conclusion

The study concludes that BHT, SMS, and citric acid in the ten selected biscuit brands were within the acceptable limit. Manufacturers of the biscuit brands studied complied with the maximum permissible limits and the acceptable daily intakes of BHT, SMS and CA in the biscuit products under investigation and the biscuits at the time of this research are safe for consumption.

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