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Microbiological and Proximate Analyses of Lebanese Bread (Pita) from Akure Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Author CEA designed the study, verified the statistical analysis and study protocol, managed the literature searches and wrote the first draft of the manuscript. Author ARF managed the analyses of the study and contributed to literature search. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at evaluating the microbial quality and proximate composition of pita samples from Akure metropolis.
Place and Duration of Study: Microbiology and Industrial Chemistry laboratories of the Federal University of Technology, Akure (FUTA), Ondo State, Nigeria, between January 2016 and June 2016.
Methodology: Pita samples were evaluated using standard microbiological techniques to ascertain microbial load and types after purchase from vendors utilizing them alone or in production of other foods. Antibiotic susceptibility profile of isolates was also determined. Proximate composition analysis of samples was also performed.
Results: Average bacterial count was 1.0±0.9 cfu/g, while average fungal count was 2.0±1.2 sfu/g. The fungi isolated were *Rhizopus stolonifer*, *Saccharomyces cerevisiae* and *Aspergillus niger*; while the bacteria isolated were *Bacillus cereus*, *Staphylococcus* sp., *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. Microbial zones of inhibition ranged from 17 mm

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to 23.50 mm. *Micrococcus luteus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were most susceptible to gentamycin, pefloxacin and amoxycillin, respectively. *Staphylococcus aureus* showed the widest antimicrobial resistant pattern. The highest carbohydrate content was recorded as 78.99 %, while bread samples showed low ash content (0.65 %).

Conclusion: Pita bread is not a common staple compared to leavened breads but provides appreciable level of nutrients. Pita bread requires regular microbial assessment to determine their safety for direct consumption or use in production of other food products.

Keywords: Antibiotic susceptibility; microorganisms; proximate; Lebanese bread.

1. INTRODUCTION

Food borne illness is a major health problem associated with foods [1,2], and results from ingestion of bacteria and/or their toxins present in foods. Food borne illnesses have a consequential impact on global economic [3]. In addition, increased multidrug resistance trends of food borne microorganisms make the global economy more vulnerable in tackling issues of food safety as it relates to public health [4]. Food borne diseases and problems relating to the sanitary and microbiological quality of foods continue to be of significant interest in Nigeria and other countries. Unfamiliar problems have been created due to development in food products, the processing and handling of foods, change of food habits and availability of convenience foods [5]. In addition, current consumer-oriented publicity on freshness of fast foods available for sale in stores has created general impression that shelf life of a food is closely related to its acceptability [5]. The question of dating and labeling of retail packages has been considered essential features of acceptable quality assurance and quality control programs. However, in Nigeria such programs are still underdeveloped. Consumers are aware of the potential for large-scale food borne outbreaks because of mishandling or improper processing of foods such breads and other pastries and demand a safer supply [5].

Bread is a common staple food. Bread production processes have evolved over the years with the discovery of fermentation and baking technologies [6]. Bread types are produced from different traditional recipes [7]. Lebanese or Pita low-fat flat bread that is slightly leavened is usually made from wheat or white flour, water, salt, sugar and yeast [8,9]. Lebanese bread can undergo chemical (hydrolytic rancidity), physical (moisture loss/gain and staling) and microbiological spoilage (water activity a_w of 0.9 to 0.96 is conducive to mould and bacterial growth) [10,11]. Bread keeping quality and shelf life studies are carried out to delay the onset of bread staleness [11,12]. Furthermore, the analytical quality control of bread and bakery foods refers to all those processes and procedures designed to ensure that the results of laboratory analysis of bread are consistent, comparable, accurate and within specified limits of precision [12].

Bakery products are an important part of a balanced diet and a wide variety of such products can be found on supermarket shelves [12]. Many industrially produced baked goods emerge from the baking process with a surface that is essentially sterile, but post bake handling can quickly lead to contamination [13]. Microbiological spoilage is often the major factor limiting the shelf life of baked products. It has been estimated that in the United States alone, losses due to microbiological spoilage are over 90 million kilograms of products each year [14]. The most principal factor influencing the microbiological spoilage of bakery products is the water activity (a_w). For low-moisture bakery products (a_w< 0.6), microbiological spoilage is not usually a problem. In intermediate moisture products (a_w = 0.65 - 0.85), osmophilic yeasts and mould are the predominant spoilage microorganisms, and a_w of Pita bread exceeds the intermediate range [15]. Yeasts which cause bread surface spoilage are mainly *Pichia burtonii* ('chalk mold'), while osmophilic yeasts contaminations occur from unclean utensils and equipment. Post baking germination of surviving bacterial spores may also initiate bread spoilage [15,16]. Therefore, maintaining good manufacturing practices will minimize contamination by osmophilic yeast and other spoilage microbes. Losses due to mould spoilage vary between 1-5% of products depending on season, type of product, and method of processing. Although fresh bread and other baked products are free of viable vegetative mould spores, products soon become contaminated from post baking contamination from the air, bakery surfaces and equipment, food handlers, and raw ingredients such as

glazes, nuts, spices and sugar [16]. Mould problems are most troublesome during the summer months due to airborne contamination and the more humid storage conditions [12]. Storage of bread under conditions of low humidity retards mould growth. In addition to the economic losses associated with bakery products, another concern is the possibility of mycotoxins production. *Eurotium* species are usually the first fungal genus to colonize. *Aspergillus* and *Penicillium* species are also known toxins producers. *Bacillus* species also bring about bacterial spoilage of bread known as ropiness which is a major economic concern in the baking industry [13].

In earlier studies, *Penicillium* species were present in nearly all loaves (stored in plastic bags for 5-6 days at 22°C) sampled within a one-year period. *Aspergillus* and *Cladosporium* species occurred in approximately half the loaves [11]. *Pichia burtonii* was also isolated and identified. Although the moisture content of Lebanese bread may be lower than that of sliced/wrapped pan bread, it's water activity (a_w) is sufficient to support moulds and bacteria growth [17]. The main moulds species that have been reported to be associated with pita spoilage are *Monilia stolonifer*, members of *Aspergillus, Rhizopus* and *Penicillium* species, particularly *Aspergillus niger*, *Penicillium expamum* and *Rhizopus stolonifer*. Al-Mohizea et al. [18] studied the shelf- life of Lebanese bread purchased from commercial bakeries in Saudi Arabia. Two to four hours after baking, the fungal and total aerobic plate counts were 6 sfu/g and 11-850 cfu/g, respectively, while coliforms were absent. Authors reported that the sanitation of bakeries varied considerably. For bread stored at ambient temperature and relative humidity (RH) of 43%, the mould free shelf-life was 9-12 days, but with *Penicillium* and *Aspergillus* species as predominant mould contaminants at RH of 39-42%. A third of isolates from the Lebanese bread samples included *Aspergillus niger*, *Rhizopus* and *Neurospora*. Mould spoilage was attributed to post-processing contamination during cooling and packaging [19,13]. Contamination can also occur from food handlers and raw ingredients such as sugar, salt, water [20].

Since foods are reported to be contaminated with microorganisms, this can hamper the health of the consumers. It was therefore important to evaluate the level of microbial contamination of baked foods such Lebanese bread in Akure to ascertain the level of safety to consumers. This study aim was to determine the microbial load and proximate composition of pita bread samples from Akure metropolis. In addition, the antibiotic resistance profiles of identified microorganisms associated with the pita bread samples were also determined.

2. MATERIALS AND METHODS

2.1 Lebanese Bread/Pita Samples Collection

Lebanese breads were obtained from three shawarma vendors at various locations in Akure. Bread samples were immediately transferred to the Microbiological Laboratory of the Federal University of Technology, Akure, Nigeria, for microbiological analysis. Proximate analysis of bread sample was done in the Department of Industrial Chemistry Laboratory of the same University.

2.2 Microbiological Analysis of Bread Samples

Total mesophilic (total viable bacterial counts) and fungi counts (yeast and mould counts) were carried out on the bread samples to determine the microbial load of the samples. One gram (1 g) of each Lebanese bread was weighed into 9 ml of sterile distilled water to make the stock solution. Thereafter, 1 ml of the stock solution was transferred into 9 ml of sterile distilled water to make the first dilution (10^{-1}), and the procedure was repeated up to dilution 10^{-4}. From the 10^{-2} and 10^{-4} dilution factor, 0.2 ml was transferred into sterile Petri dishes. Approximately 20 ml of molten Nutrient agar (NA), Potato Dextrose agar (PDA) and Eosin Methylene Blue (EMB) agar were transferred into the respective labelled petri plates seeded with microorganism. The plates were then gently rocked to allow even distribution of the organisms. Afterwards, the media were left to solidify, and Nutrient agar and Eosin methylene blue agar plates were incubated at 37°C for 24 h for bacterial growth, and Potato Dextrose agar plates at 28 ± 2°C for 3-5 days to allow for fungal growth. Bacterial counts were expressed in colony forming unit (cfu) per gram and spore forming unit (sfu) per gram for the fungi. All counts were done in duplicate. Observed colonies were sub cultured repeatedly on media used for primary isolation to obtain pure cultures [21].

2.3 Characterization and Identification of Isolates

Representative colonies from the incubated plates were picked and streaked on separate sterile plates of solidified medium. The plates were incubated at 37°C for 18-24 h. This was repeated until a pure culture was obtained. A loopful of the inoculum was preserved on agar slants of double strength and incubated at 37°C for 24 h. The slants were stored in the refrigerator at 4°C.

The bacterial isolates were characterized using Gram reaction and biochemical tests (catalase, spore, coagulase, urease, triple sugar iron (TSI), and identified by comparing their characteristics with those of known taxa as outlined in Fawole and Oso [21] and Bergey's Manual of Systematic Bacteriology [22,23]. The fungal isolates were characterized based on macroscopic and microscopic (stereomicroscope) examination and identified according to Olutiola et al. [24].

2.4 Antibiotics Sensitivity Test

The identified microbial isolates were screened for antibiotics susceptibility patterns using the disc diffusion method of Bauer et al. [25], and 0.5 McFarland turbidity standard for broth cultures. Using an aseptic technique, a sterile swab was placed into the broth culture of a specific organism. The swab was then used to streak the surface of appropriately labelled Mueller Hinton agar plates to form a bacterial lawn and plates allowed to dry. The antibiotics used included: Augmentin (30 µg), Gentamycin (10 µg), Ofloxacin (5 µg), Amoxycilin (25 µg), Ciprofloxacin (10 µg), Amoxycillin (25 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Pefloxacin (5 µg), Erythromycin (5 µg), Zinnacef (20 µg), Rocephin (30 µg), Septrin (30 µg). Each antibiotic disc was gently pressed to the inoculated agar surface using a flame-sterilized forceps to ensure that the disc was attached to the agar. Plates were then incubated overnight at 37°C. Thereafter, the inhibition zone diameter was determined by measuring along two axes, that is at right angles to each other, for each zone and the mean calculated and recorded to the nearest millimetre (mm). The cut off limit used for antibiotic susceptibility was ≥15 mm [25].

2.5 Proximate Evaluation of Pita Bread

2.5.1 Moisture

Moisture content, as well as other proximate parameters of the bread, was determined using the procedure described by AOAC [26], and calculated using:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (1)$$

Where; W_1 =weight of empty evaporating dish, $W_{2=}$ weight of sample + evaporating dish, $W_{3\,=}$ weight of sample + evaporating dish after drying at 105°C.

2.5.2 Crude protein

The concentration of protein in the digested sample was determined spectrophotometrically and calculated as:

$$\% \text{ crude protein} = \frac{(\text{titre of sample} - \text{blank}) \times 0.01 \times 14.007 \times 6.25 \times 100}{10 \times \text{weight of sample}} \quad (2)$$

2.5.3 Crude fat

Crude fat content of samples were calculated using the formula:

$$\% \text{ Fat} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1} \quad (3)$$

Where;

W_1 =weight of empty extraction thimble, $W_{2=}$ weight of sample + extraction thimble, $W_{3\,=}$ dried weight of defatted sample + extraction thimble.

2.5.4 Crude fibre and ash content

The percentage crude fibre and ash content of Pita samples were calculated as shown below:

$$\% \text{ Crude fibre: } \frac{W_2 - W_3}{W_1} \times \frac{100}{1} \quad (4)$$

Where; W_1 =weight of defatted sample
$W_{2=}$ weight of sample at 105°C
$W_{3\,=}$ weight of sample at 550°C

The percentage ash content was calculated using the formula:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} \quad (5)$$

Where; W_1 =weight of empty crucible, $W_{2=}$ weight of sample + crucible, $W_{3\,=}$ weight of sample + crucible after ashing at 550°C.

2.5.5 Available carbohydrate

The available carbohydrate was determined after subtraction of other predetermined proximate

parameters such as crude fibre, crude protein, and fat content. The remaining accounted for carbohydrates:

% available carbohydrate = 100 – (% protein + % fat + % ash + % moisture + %crude fibre) (6)

2.6 Statistical Analysis

Experiments were carried out in triplicates. The data obtained were analysed by one-way analysis of variance (ANOVA) and the means were compared by New Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Average Counts and Identities of Bacterial and Fungal Isolates

The mean bacterial and fungal counts were presented in Table 1. The average fungal count was 2.0±1.2 sfu/g while the average bacterial count was 1.0±0.9 cfu/g. No growth was observed on Eosin Methylene Blue (EMB) agar plate. The identity of the bacteria isolated from the bread samples were shown in Table 2 and include *Staphylococcus aureus, Bacillus* sp., *Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Proteus vulgaris* and *Corynebacterium* sp. Fungal species identified included *Saccharomyces cerevisiae, Rhizopus stolonifer* and *Aspergillus niger* (Table 3).

Bread is a good growth medium for many microorganisms because of its near neutral pH, and availability of essential nutrients for microbial growth and multiplication [5,12]. The presence of microorganism of food and health importance in our pita bread samples could be indicative of improper handling, poor equipment sanitization and unhygienic production environment. Food handlers serve as sources of contamination for the product [27]. Equally important is the bread holding temperature and length of time the bread is stored before testing and processing. Any surviving bacterial spores may germinate within this period [27]. All these factors could influence the total microbial count and the types of microorganism present in the bread [5,27].

During sample collection it was observed that pita vendors payed little or no attention to hygiene. Bread products were kept under unhygienic conditions, and hand washing was rarely practised. This may have contributed to the presence of *Staphylococcus aureus* in the samples. Contamination with *Staphylococcus aureus* which had a higher percentage of occurrence in all food samples may have resulted from pre- and post-cooking handling of the foods [28]. This bacterium makes up to 25% of normal skin flora of healthy humans and can produce heat resistant toxins [29,30]. According to Vijay et al. [31], Staphylococcal food poisoning caused by enterotoxin-producing strains of *S. aureus*, was the most common food borne illness. Toxin producing strains of *Staphylococcus* are the leading cause of gastroenteritis following poor and unhygienic handling of food products. Cooking utensils used are often contaminated with *Micrococcus luteus*. In this study it may have originated from the hands when they touch the food preparation areas, pans, clothes or water during pan and hand washing. In other words, cross contamination between pan washer, food preparation surface and the food itself is possible [32].

The presence of *Bacillus* sp. also indicated poor sanitary conditions during handling, and transportation of foods. Dierick et al. [33] reported that *Bacillus* sp. spores adhere to many surfaces and survive normal washing and disinfection (except for hypochlorite and ultraviolet C) procedures. Aruwa and Akinyosoye [34] also recently reported the presence of *Bacillus* sp. in analysed food products and pastries. *Proteus vulgaris* is also a common inhabitant of the human gut and a urinary tract pathogen which could be present in the food through unhygienic practices [35]. The presence of *Saccharomyces cerevisiae* in the bread samples may be attributed to the use of *Saccharomyces cervisiae* (bakers' yeast) in the leavening of pita bread samples [4,36]. The presence of *Aspergillus* sp. fungus could have detrimental effects on humans as several species have been associated with toxin production which cause diseases known as aflatoxicoses [37]. Ijah et al. [38] had also earlier reported the presence of *Aspergillus* and *Rhizopus* species in bread samples made from wheat/potato flour blends. In addition, we note that while pita aerobic bacterial counts was within acceptable limit of $<10^5$ cfu/g), the mean aerobic fungal counts (2.0±1.2 sfu/g) were within unsatisfactory microbiological limit of $\geq 10^5$ sfu/g [39]. Therefore, food products analysis should be encouraged locally and globally to ensure

aerobic counts fall within acceptable limits for food products consumption in order to safeguard public health [39].

3.2 Antimicrobial Susceptibility Patterns of Isolates

The zone of inhibition of Gram negative and Gram-positive isolates in response to antibiotics were shown in Table 4. The inhibition zones for Gram positive bacteria ranged from 17.00-23.50 mm. *Micrococcus luteus* was more susceptible to gentamycin with zone of inhibition of 23.50 mm, *Staphylococcus aureus* was more susceptible to pefloxacin with zone of inhibition of 23.50 mm, while *Staphylococcus epidermidis* was more susceptible to gentamycin and rocephin with zone of inhibition of 23.00 mm each. *Corynebacterium* sp. was more susceptible to rocephin and septrin with zone of inhibition of 21.50 mm. *Bacillus cereus* was more susceptible to rocephin, pefloxacin and streptomycin with zone of inhibition of 22.50 mm. Pefloxacin was the most effective of the antibiotics against the Gram-positive isolates. Antibiotic zones of inhibition for Gram-negative isolates was shown in Table 4, with inhibition zones ranging from 12.50-23.50 mm.

In the antibiotic susceptibility test, identified isolates showed variable susceptibility/resistance patterns. *Micrococcus luteus* was resistance to most of the antibiotics. This finding probably links the source of these isolates which could be humans commonly colonized by multidrug resistant bacteria. The finding is similar to the report of Udo et al. [40], who reported the antimicrobial resistance profile of potential human pathogens isolated from fresh fast food samples in Calabar, Nigeria. Their findings were linked to the acquisition of multidrug resistance mechanisms which may be intrinsic or extrinsic. In this study, all bacterial isolates were susceptible to pefloxacin and streptomycin with distinct zones of inhibition [40]. In other words, it may be inferred that pefloxacin and streptomycin were highly effective in inhibiting these bacteria antibiotic resistance mechanisms. *Staphylococcus aureus* and *Proteus vulgaris* were resistant to a wide variety of antibiotics. This may be linked with the inability of the antibiotics to permeate the cell wall/membrane or inhibit the microorganisms' protein synthesis pathways [41]. *Micrococcus luteus* was susceptible to all the antibiotics with wide zones of inhibition. This may be attributed to the efficient diffusion of antibiotics into the semipermeable cell wall/membrane of the microbe [41]. The wide antibiotic resistance profile of *S. aureus* and some other identified isolates in this study calls for serious concern. Control and preventive protocols to reduce the spread of antibiotic resistance need to be put in place and enforced nationally and internationally. This would ensure the continued efficacy of existing antibiotics in treatment of food borne illnesses.

3.3 Proximate Composition of the Bread Sample

The proximate composition of the bread samples were presented in Table 5. Mean carbohydrate content of sampled breads was the highest (78.99%), while ash content (0.65%) was the lowest of all the proximate parameters measured. There was no significant difference between the mean ash content and mean crude fibre of pita samples. Likewise, no significant difference was observed between the mean moisture and fat contents, but significant difference observed for ash, fat, protein and carbohydrate contents.

Moisture content of food products is a major determinant of a food product's shelf life. Moisture content of pita breads sampled in this study (5.15%) were within acceptable limit for dry food products (15%) [42]. On the contrary, Ethiopian kitta flat breads made from salt, water, and different ratios of maize and sweet potato flour blends showed 20.96-28.95% moisture content range. High moisture values were attributed to high water binding capacity of the starch in the sweet potato flour [42]. Similar observation of higher moisture content was recorded (11.50-15.31%) for leavened bread samples made from wheat and potato flour blends, water, sugar, milk, salt and yeast, which could be attributed to the addition of milk and other liquid ingredients added during bread production [38]. The lower the moisture content of a bread product, the higher probability of a longer the shelf life. In this study, fat content (4.70%) however fell within the range reported for Kitta flat breads (4.42-5.17%) [42], but higher than for bread made from 100% wheat (2.67%) [38]. Variations may be due to processing methods and bread making ingredients used.

Table 1. Enumeration of total microbial counts

Isolation medium	Average bacterial count (x10^4 cfu/g)	Average fungal count (x10^5 sfu/g)
Eosin methylene Blue (EMB)	No growth in samples	Not determined
Potato dextrose agar (PDA)	Not determined	2.0 ± 1.2
Nutrient agar (NA)	1.0 ± 0.9	Not determined

Table 2. Biochemical characteristics of bacteria isolated from the Lebanese bread samples

Tests	Isolate						
	1	2	3	4	5	6	7
Gram reaction	+	+	+	-	+	+	-
Catalase	+	+	+	+	+	+	+
Coagulase	-	+	-	+	+	-	+
TSI	A	A	A	A	A	A	A
Lactose	-	A	A	-	-	-	A
Glucose	A	A	A	A	A	A	A
Sucrose	-	A	A	A	-	A	A
Spore	-	-	-	-	-	+	-
Urease	ND	ND	ND	+	ND	ND	+
Suspected organisms	*Micrococcus luteus*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Proteus vulgaris*	*Corynebacterium* sp.	*Bacillus* sp.	*Pseudomonas aeruginosa*

A= Acid production, + = positive, - = negative, ND = Not determined, TSI = Triple sugar Iron

Table 3. Characteristics of fungi isolated from Lebanese bread samples

S/N	Macroscopic	Microscopic	Probableisolates
1	Colonies grow rapidly within 3 days, yellow to creamy colour, dull and smooth.	Presence of ellipsoidal cell buds, and pseudohyphae. Hyphae absent.	*Saccharomyces cerevisiae*
2	Colonies with fluffy and velvety texture. White mycelium developing to brown then black conidial heads.	Globose conidia head. Septate hyphae. Conidiophores arising from the substratum. Phialides borne on roundswollen vesicles.	*Aspergillus niger*
3	Colonies growth spreads rapidly within 2-3 days. Cottony white mass of mycelium, with black spores. Differentiated stolons, terminating in tufts of rhizoids.	Sporangiophores unbranched and erect. Sporangia globose. Spores variously shaped, striated in long axis.	*Rhizopus stolonifer*

Table 4. Antibiotics sensitivity test on gram-positive and gram-negative bacteria isolates (mm)

Isolates	Antibiotics (Gram-positive)									
	CIP	**STRP**	**SEP**	**ERY**	**PEF**	**GEN**	**AMP**	**ZIN**	**AMO**	**ROC**
B1	23.00±1.00^{d}	17.50±0.50^{b}	21.50±0.50^{d}	18.00±1.00^{a}	21.50±0.50^{b}	23.50±0.50^{d}	21.00±1.00	19.50±0.50^{a}	18.00±0.00^{a}	20.50±0.50^{a}
B2	-	17.50±0.50^{b}	-	-	23.50±0.50^{d}	-	-	-	-	-
B3	20.50±0.50^{b}	18.00±1.00^{c}	19.00±1.00^{c}	20.00±0.00^{b}	21.00±1.00^{b}	23.00±1.00^{d}	-	20.50±0.50^{b}	-	23.00±1.00^{d}
B5	20.50±0.50^{b}	18.50±0.50^{c}	21.50±0.50^{d}	20.00±0.00^{b}	20.50±0.50^{a}	17.00±1.00^{b}	-	-	19.00±1.00^{b}	21.50±0.50^{b}
B6	20.50±0.50^{b}	22.50±0.50^{e}	18.50±0.50^{b}	20.50±0.50^{b}	22.50±0.50^{c}	18.50±0.50^{c}	-	20.50±0.50^{b}	-	22.50±0.50^{c}
	ANTIBIOTICS (Gram-negative)									
	CIP	**STRP**	**SEP**	**CHL**	**PEF**	**GEN**	**AUG**	**OFL**	**AMO**	**SPAR**
B4	17.50±0.50^{a}	20.50±0.50^{d}	-	-	22.50±0.50^{c}	11.50±0.50^{a}	-	20.50±0.50^{a}	-	-
B7	21.50±0.50^{c}	12.50±0.50^{a}	15.00±1.00^{a}	20.50±0.50	20.00±0.00^{a}	17.00±1.00^{b}	18.50±0.50	20.50±0.50^{a}	23.50±0.00^{c}	-

Data are presented as Mean ± standard deviation (SD) (n=3). Values within a column bearing different superscript letters for same antimicrobial agent/compound are significantly different (P= .05). CIP= Ciprofloxacin (10µg); STRP = Streptomycin (10µg); SEP = Septrin (30µg); ERY = Erythromycin (5µg); PEF = Pefloxacin (5µg); GEN = Gentamycin (10µg); AMP = Ampliclox (30µg); ZIN = Zinnacef (20µg); AMO = Amoxycillin (25µg); ROC = Rocephin; CHL = Choramphenicol (30µg); AUG = Augmentin (30µg); OFL = Ofloxacin (5µg); SPAR = Sparfloxacin (10µg). Gram positive: B1 = Micrococcus luteus; B2 = Staphylococcus aureus; B3 = Staphylococcus epidermidis; B5 = Corynebacterium Sp.; B6 = Bacillus cereus. Gram negative: B4 = Proteus vulgaris; B7 = Pseudomonas aeruginosa

The average fibre content of our pita bread samples (1.39%) was within the 1.5% maximum allowable fibre content of bread flour [43] but were lower than the range recorded for kitta breads (2.08-3.51%) from different flour ratio/blends. A high food fibre content may make food harder to digest and the level of food nutrients absorption into the body may also be affected [42]. In addition, their report of a higher kitta fat content value was attributed to the use of two flour blends [42]. Our pita samples mean protein content (10.12%) differed significantly from those of kitta breads (7.56-8.94%) [40] and 100% wheat breads (12.25%) [38]. Protein content of food products may be linked to the type of food components utilized in making the products [38]. High carbohydrate content was recorded for our pita bread samples (78.99%). Tadesse et al. [42] reported lower (55.99-61.35%) carbohydrate content in flat Ethiopian kitta bread. Variations may be attributed to processing methods used and quantity of ingredients used in bread making. Carbohydrates are desirable in baked products. This is because they form starch granules on heating in water, swell and become gel-like. The gel contributes to the characteristic structure and texture of the final baked product [44].

Table 5. Proximate composition of pita bread samples

Proximate parameter	% composition
Ash	0.65 ± 0.00^{a}
Moisture	5.15 ± 0.08^{d}
Crude fat	4.70 ± 0.19^{c}
Crude protein	10.12 ± 0.02^{e}
Crude fiber	1.39 ± 0.24^{b}
Available carbohydrate	78.99 ± 0.95^{f}

Data are presented as Mean±SD (n=30). Values in a column bearing different superscript letters are significantly different (p<0.05)

4. CONCLUSION

Pita bread is rich in basic food nutrients such as carbohydrate and protein. The common microorganisms associated with pita bread are bacterial and fungal species of food and medical importance. The presence of microbial load that is higher than stipulated microbiological limit, and the possibility of having pathogens with wide antibiotic resistance profiles serve as pointers to warn that care should be taken in order to avoid foodborne poisoning from multidrug resistant microbial strains consumed from pita bread. In addition, public health is a global concern. Therefore, the consumption of microbiologically safe pita cannot be overemphasized.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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