RESEARCH ARTICLE

ISSN: 0331 - 670X
https://doi.org/10.51412/psnnjp.2021.15

The Nigerian Journal of PHARMACY
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Bacteriological examination and potassium bromate analysis of bread samples from selected retail outlets in two locations south west Nigeria

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ARTICLE INFO

Article history:

Received	15 May 2021
Revised	3 July 2021
Accepted	8 Aug 2021
Online	30 Sept 2021
Published	-

Keywords:

Bacteriological examination,
Potassium bromate,
Bread,
Ibadan,
Sagamu.

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ABSTRACT

Background: Bread is a staple food prepared from dough of flour and water, usually by baking. It is one of the oldest man-made foods consumed by all socio-economic groups. This study was carried out to evaluate the bacteriological profiles and investigate the potassium bromated additives in bread from retail outlets in south west Nigeria.

Methods: Bread samples homogenates were examined by standard plate count method on different culture media for isolation of *Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa, Penicillium spp*. And *Rhizophus spp* were also analyzed for the presence of potassium bromate by spectrophotometry. Potassium bromated in the bread samples was quantitively and qualitatively analyzed using spectrometric determination of bromated in bread.

Results: All the 10 samples were found to contain bacteria and molds in varied proportions. Average counts of the isolates for both packed and unpacked bread samples; *Staphylococcus aureus*,(2.6×10^{4}cfu/g): (4.0×10^{5}cfu/g), *Escherichia coli* 1.08×10^{3}cfu/g : 1.66×10^{5}cfu/) *Pseudomonas aeruginosa* (0.79×10^{2}cfu/g): (1.40×10^{5}cfu/g), *Penicillium spp* (1.92×10^{4}cfu/g): (2.74×10^{5}cfu/g) and *Rhizopus* (1.92×10^{3}cfu/g): (2.71×10^{5}cfu/g)respectively while the bromate levels ranged from*0.02 ± 0.05 ppm to 9.33 ± 0.30 ppm were, an indication of not all bakeries following the standard regulation required by NAFDAC for bread production.*

Conclusion: The presence of food poisoning bacteria in the bread examined could be traced to raw material quality, sanitary status of equipment, handlers hygiene, environment and storage condition while the amount of potassium bromate content found could threaten consumers' life when consumed regularly and hence, the needs for strict monitoring by the food and drug regulatory agency

1. Introduction

Bread is a food product that is universally accepted as a very convenient form of food that has desirability to all population rich and poor, rural, and urban. Its origin dates to the Neolithic era and is still one of the most consumed and acceptable staples in all parts of the world. Statistical analysis in Nigeria showed that bread is one of the most consumed food types in homes, restaurants, and hotels with predominant consumption among the poor and young ones who constitute more than 75% of the overestimated 180 million people in Nigerian[1.]

Bread is made from low protein wheat flour. Some of the basic ingredients, apart from flour, are table salt, sugars, flavors', and at least, a flour improver such as potassium. Bread are named after the ingredient in their composition and baking process; whole meal bread, brown loaf, dark rye bread, corn bread, pita, oatmeal soda bread, sweet cherry bread, apricot and hazelnut bread, black olive bread, roasted red onion bread and toasted seed and nut bread[2]

Bread can be leavened chemically by adding baking powder to the bread dough or microbiologically by adding cultures of the yeast *Saccharomyces cerevisiae.* Both methods result in the evolution of carbon dioxide which makes the bread rise. However, a taste of the products of each of these methods gives evidence that the activities of the yeast improve the texture of bread and yield aromatic compounds that greatly enhance the flavor of bread. The alcohol produced along with CO_2 vaporizes and escape during baking[3]

Everyone has experienced the annoyance of opening a loaf of bread and finding it covered with black "whiskers" of bread mold. These kind of spoilage result from invasion of food, by species of *Rhizopus* on the bread. Bread and other bakery products are subjected to various spoilage problems, viz., physical, chemical and microbial; the latter is the most serious one particularly bacterial (*Bacillus* sp.) and mold growth. Various molds involved in spoilage of bread include *Rhizopus, Mucor*, *Penicillium*, *Eurotium*, *Aspergillus* and *Monilia.* Likewise, yeast spoilage known as "Chalk mold" is caused by *Pichia butonii*[4]

The retailers with little or no knowledge about the fundamental of personal hygiene, the storage and preservation methods, could be a source of contamination to packed and unpacked bread most importantly potassium bromate, ($KBrO_3$) is an oxidizing agent that are used by bakers to improve dough texture and enhance bread quality. KBrO3 acts as a maturing agent and dough conditioner by oxidizing the sulfhydryl groups of the gluten protein in flour into disulphide bonds making it less extensible and more elastic; this makes the dough visco-elastic and equipped it to retain the carbon dioxide produced by the yeast. It causes flour maturation and strengthens the gluten network thereby improving the product volume [5]

The international agency for research on cancer classified potassium bromate $KBrO_3$ as a category 2B carcinogen in 1986, based on sufficient evidence that KBrO3 induces cancer in experimental animals and may (possibly carcinogenic to humans). This finding led to the ban of bromate in flour and bakery products by many countries of the world. The maximum concentration of potassium bromate allowed in bread by the US Food and Drug Agency (FDA) is 0.02 μg/g (0.02 mg/kg)[6]

In Nigeria, the use of potassium bromate in bread and related products was banned in 1993. Twenty-eight years after ban, the compliance with this regulation is extremely low in many bakeries across the country. Therefore, this study was carried out to evaluate the bacteriological profiles and investigate the potassium bromate additives in bread from selected retail outlets in Ibadan and Sagamu axis, located in south west Nigeria.

2. Methods

2.1 Collection of samples

Bread samples were purchased from retail outlets in New Garage, Toll gate road junction Ibadan situated between the latitude 7.3964 and longitude 3.9167 in Oyo state and Awolowo market and Total Express junction axis in Sagamu, located within the latitude 6.832201 and longitude 3.631913 in Ogun state southwest Nigeria. Both packed and unpacked sample was collected in a sterile sealed polythene bag, appropriately labeled and immediately brought to the laboratory for bacteriological and spectrophotometric analysis.

2.2 Microbiological evaluation 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 as described by American Public Health Association[7] Bread samples were prepared by mashing and mixing the powdery crumb in peptone water and were diluted decimally. A volume of 0.1 mL aliquots were spread plated on nutrient agar (NA), MacConkey agar (MCA), mannitol salt agar (MSA) and Saboraud dextrose agar (SDA) for the enumeration of aerobic viable bacteria and fungi, respectively. The NA and MCA plates were incubated at 37^{o}C for 24–48 hours while SDA plates were incubated at room temperature (25 ± 28^{o}C) for 3–5 days. The biochemically identified colonies were then counted and expressed as colony forming units per gram (cfu/g) of samples. All counts were done in duplicate using the Quebec colony counter. Distinct colonies were sub cultured repeatedly on media used for primary isolation to obtain pure culture.

2.3 Determination of potassium bromate

Potassium bromate in the bread samples was qualitatively and quantitatively analyzed using methods of Ojeka *et.al*.[8] on spectrophotometric determination of bromate in bread by oxidation of dyes. A quantity of 1.0 g was weighed out from each bread sample in an electronic weighing balance. This was transferred into a test tube. 10 ml of distilled water was added; the mixture was shaken and allowed to stand for 20 minutes at 28 ± 10 °C. A 5.0 mL volume was decanted from the test tube. A 5.0 mL volume of freshly prepared 0.5 % potassium iodide solution in 0.1N hydrochloric acid was added. And color changes were noted. The presence of potassium bromate was indicated by change in colour from light yellow to purple. The absorbance of the sample was taken at 620 nm in a colorimeter (SM-Spec. 7504). Absorbance of the sample was converted to concentration with reference to Beer's calibration curve previously constructed for potassium bromate using the pure sample and the mean of five replicate determined are presented[8].

3. Results

Bread samples were visually examined on their retail sales location mostly beside major roadside, some are left unpacked, some are covered with tarpaulin and or women wrapper, some are with dirty nylon pack, some are with sweated nylon pack with condensation of moisture as a result of been left in the sun, few are looking fresh, some are looking blistered, some are smelling yeast, some are roughly looking and unappealing, some are whitish, bumpy and poorly corrugated, some are with labels while most are with no label nor with NAFDAC registration number or questionable uncompleted NAFDAC number and the sellers are not all hygienically dressed.

Table 1: Identity, sizes and forms of selected bread samples

Description code (loaf Identity)	Loaf Value Size(g)	Loaf type	Manufacturer specification	Packed/Unpacked	NAFDAC Reg. number
A	80	Sliced	Bromate free	Packed	Registered
B	100	Unsliced	Bromate free	Packed	Registered
C	100	Unsliced	No information	Unpacked	NNN -
D	100	Unsliced	Bromate free	Unpacked	NNN
E	50	Unsliced	Bromate free	Unpacked	NNN
F	100	Sliced	No information	Unpacked	Registered
G	50	Unsliced	No information	Unpacked	NNN
H	100	Unsliced	No information	Unpacked	NNN
I	50	Unsliced	No information	Unpacked	NNN
J	50	Unsliced	No information	Unpacked	Registered

Key: A - J code are the identity of the bread samples examined.

NNN – No NAFDAC Number

The standard plate counts of the isolates of bacterial and molds obtained on different culture media showed variation of microbial load for each selected dilutions for both packed and unpacked breads and their average dilutions were recorded as shown in Table 2 and 3 respectively

Table 2: Microbial load (cfu/g) in packed bread samples

Bread sample	*S. aureus(cfu/g)*	*E. coli(cfu/g)*	*P. aeruginosa (cfu/g)*	*Penicillium spp (cfu/g)*	*Rhizopus.spp (cfu/g)*
A(packed)	1.0×10^3	0	0	0	0
B(packed)	1.0×10^3	0	0	0	0
C(packed)	3.2×10^4	1.6×10^5	1.2×10^3	3.4×10^6	3.2×10^5
D(packed)	4.0×10^6	2.0×10^5	1.52×10^5	5.2×10^6	5.0×10^6
E(packed)	3.8×10^6	1.8×10^5	1.23×10^4	1.0×10^6	1.4×10^5
Average	**2.6×10^4**	**1.08×10^3**	**0.79×10^2**	**1.92×10^4**	**1.92×10^3**

Table 3: Microbial load (cfu/g) in unpacked bread samples

Bread Sample	*St. aureus(cfu/g)*	*E. coli(cfu/g)*	*P.aerug.(cfu/g)*	*Penicilliums spp (cfu/g)*	*Rhizopus.sp p(cfu/g)*
F(unpacked)	2.8×10^5	1.60×10^5	1.2×10^5	1.2×10^3	1.0×10^3
G(unpacked)	3.0×10^3	1.22×10^5	1.3×10^5	1.52×10^5	1.2×10^5
H(unpacked)	4.2×10^4	1.40×10^5	1.52×10^3	3.7×10^6	3.6×10^5
I(unpacked)	4.8×10^6	1.80×10^5	1.72×10^5	5.5×10^6	5.2×10^6
J(unpacked)	5.2×10^6	2.3×10^5	1.25×10^6	1.8×10^6	1.55×10^4
Average	**4.0×10^5**	**1.66×10^5**	**1.40×10^5**	**2.74×10^5**	**2.71×10^5**

Ten (10) bread samples analyzed in this study contained potassium bromate in varying quantities. Sample A had the least content of$KBrO_3$ (1.02µg/g) while sample-J had the highest concentration of $KBrO_3$ at 10.33 µg/g as shown in Table 4 The quantity of $KBrO_3$ in each bread sample correlates with the degree of color obtained in the qualitative test as specified in Beer Lambert's law.

Table 4: Qualitative and quantitative determination of potassium bromate of the ten selected bread samples

Values are presented as mean ± standard deviation with n = 5

Bread sample	Color Reaction With Potassium Iodide (Qualitative Test)	Concentration of Potassium Bromate (µg/g) (Quantitative Test)
A	No visible color change	0.02± 0.05
B	No visible color change	1.05± 0.03
C	Purple	3.22 ± 0.31
D	Purple	4.18 ± 0.22
E	Light Purple	4.82 ± 0.24
F	Purple	5.48 ± 0.37
G	Purple	6.82 ± 0.16
H	Purple	8.03 ± 0.33
I	Purple	8.00 ± 0.81
J	Purple	9.33 ± 0.30

4. Discussion

Ten randomly sampled were examined for the presence of microbial loads which may probably be as a result of erroneous handlings, hygiene status, and storage condition the bread samples were subjected to on their retail sites. On-site visual assessment of the bread showed that 60% of the bread sampled did not have NAFDAC registration number, the specification on their labels that they are bromine free which was found to be untrue in almost all the bread sampled, the bacterial and moulds distribution varied from one bread to another which was similar to the study of Lukman *et.al.,*[9] on determination of potassium bromate content in selected bread samples in Gwagwalada,Abuja-Nigeria[9.]

The loaf sizes imprinted on 60% of the bread samples as their weight are not true and 40% of the sampled labeled bread elicited blurred, incomplete questionable NAFDAC registration numbers which seems to be intentional after day-by-day routine inspection probably to hide their location from NAFDAC tracking. The costs of the bread sampled depended on their sizes and quality.

The average counts of *Staphylococcus aureus* for both packed and unpacked samples was the highest; 2.6×10^6 and 4.6×10^6 respectively while *Pseudomonas aeruginosa* had the lowest microbial counts in both packed and unpacked samples;0.79×10^4 and 1.40×10^5 as shown in Table 2 and 3 respectively. This is beyond $\leq10^3$cfu/g for bacterial and $\leq10^5$ for yeast international microbiology standards recommended units for ready to eat food. Moulds (*Penicillium and Rhizopus spp.)* obtained from 3 of the 10 sampled bread from this study has potential of producing mycotoxin in concentration capable of causing food deterioration or food borne illness, since the recommended limit for yeast and moulds in bread is 10^5cfu/g. Sample A and B(packed) in this study were void of other microbes but with little presence of *Staphylococcus aureus* which could be due to contamination from bakery or handling error .Though, it required high dose of *Staphylococcus aureus* (10^5 – 10^6cfu/mL) to cause food poisoning. This agreed with the study of Tabashsum *et.al*.,[10] on prevalence of spoilage microorganisms in different street food in Dhaka city[10.]

Potassium bromate has been used as a dough conditional for the past 6 decades. According to the United States Department of Agriculture (USDA), it improves dough processing properties, internal crumb quality and low volume in concentration from a few to 75 ppm. In early 1990's, the World Health Organization (WHO) discovered that potassium bromate if consumed has the capacity to cause such diseases as cancer, kidney failure and several other related diseases. And the Joint FAO/WHO committee's recommendation of acceptance level of 0–60 mg KBrO3 bread was withdrawn because of long term toxicity and carcinogenicity[11].

One disappointing but contradictory finding in this study was that the bread samples with "bromate free" in their labels contained potassium bromate in varying concentrations than those without such inscription on their labels. The potassium bromate obtained in this study ranged from *0.02 ± 0.05* **(µg/g)** *to 9.33 ± 0.30* **(µg/g)** which has exceeded (0.02 mg/kg) the maximum concentration of potassium bromate allowed in bread by the Food and Drug Agency (FDA) which was similar to the study of Nakamura[12] on the effect of reducing agents and baking conditions on potassium bromate in bread[12]. With respect to bromate content, two of the ten bread samples analyzed (sample A and B) has low bromate content though its consistent consumption in bread could be life threatening.

5. Conclusion

The presence of food bacteria and molds found on the bread examined in varied proportion in this study could be traced to raw material quality, sanitary status of equipment, handlers' hygiene, environment, and storage condition while the amount of potassium bromate content found could be due to profit making drive of the bakers to outsmart the Covid-19 mediated astronomical increase in prices of ingredients and raw materials. Therefore, there is need for government to ensure the ease of getting the required ingredients at affordable prices and to fund and equipped food and drug regulatory agency for effective monitoring of bakeries to enforce rules and regulations as applied to bread and baked products.

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