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Microbial analysis of three types of bread sold in Sokoto Metropolis

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ABSTRACT: Pooled of 300 samples, 100 each of yellow, chocolate and white bread were microbially analyzed to detect the level of contamination and spoilage organism. The following bacteria were isolated, *Staphylococcus saprophyticus, S. aureus, Bacillus subtilis, B. alvei, B.licheniformis, Cornebacterium* sp and *Proteus* sp from 230 loaves. The fungi isolated were, *Aspergillus niger A flavus, Mucor mich, Aspergillus oryzae, R. stolonifer* and *Penicillium* sp. The percentage occurrence in each bread were 70%, 60% and 100% in yellow, chocolate and white bread respectively.

Key Words: Bread; Food spoilage organisms; Microbial contamination.

Introduction

Essential nutrient necessary for the body are present in the food. Various types of foods are available and sources, mode of preparations as well as method of consumption differ. Some are consumed by chewing, some by eating in solid form and some may be in liquid form (1).

Bread is widely eaten and considered to be one of the fast food, it provides a larger share of peoples energy than any other food (2).

Bread is made by baking dough that consists chiefly of flour or grain meal mixed with water and milk prepared in most parts of the country by hand.

Flavours ar used during baking of bread Flavouring substances so developed may include alcohol, diacetyl, aldehydes, acetone, isoalcohols and lactic, acetic and succinic acids and their esters (3).

The microbial quality of food in its raw or unprocessed state, the sanitary conditions during handling, packaging, storage and the environment of the food are the parameters that determine microbial numbers and types of products (4).

Bread, because of its nutritional nature, is highly susceptible to heavy microbial contamination and possible involvement in food poisoning. Because of its method of packaging which is mainly by hand, possible contamination with food poisoning organisms such as *Staphylococcus aureus* which is often found in the human hands (5) and the forming bacteria and molds which produce toxic metabolite which can seriously affect the quality of bread resulting in health hazard to the consumers.

This work, therefore, is aimed at isolating microorganism present in yellow, chocolate and white bread and also to assess the bread for microbial load.

Materials and Methods

The bread samples used for this research were purchased from different bread retailers in different parts of Sokoto metropolis and transported immediately to the microbiology laboratory of the Usmanu Danfodiyo University, Sokoto.

All the glass ware used were thoroughly washed with detergents and sterilized in hot air oven at 160°C for 1 hour.

Nutrient and Saboraud agar used in the research were prepared according to the manufacturer's specification.

Bread analysis

10.0gms of each sample was collected and transferred into Universal bottle containing peptone water and incubated for 4 hours. Serial dilution was carried out by pippeting 1.0ml from the stock bottle and transferred to test-tube containing 9.0ml of distilled water. This bottle was marked 10^1. Similar procedure were carried out to 10^4 media inoculation was done on 10^4 dilution.

Media Inoculation

Aliquots of 0.1ml of the diluent from 10^4 test-tube was transferred to the Petri-dishes with a sterilized bent glass rod (in alcohol). These were then incubated at 37°C for 24 hours.

For fungi isolation, 10.0g of the bread sample was transferred into Petri-dishes containing Saboraud dextrose agar incubated at room temperature (27°C) for four days.

Sub-culturing

The suspected colonies after stained were subculture on another fresh nutrient agar plate pure culture. Confined, purified isolates were transferred into agar slant to serve as stock cultures stored in a refrigerator at 0 – 8°C. From this stock the microorganism were identified.

Identification of bacterial isolate

Characteristics of bacteria were considered and these include macroscopic (cultural) and microscopic appearances, biochemical and carbohydrate fermentation.

Biochemical tests were performed, also performed were citrate utilization, urease and Triple sugar tests.

Results

The results of the study shows that of 300 samples analysed, 100 samples each of white, yellow and chocolate, 230 samples had bacterial growth and all the samples gad fulgal growth on them.

Yellow bread had 1.26 x 10^6 cfu/ml respectively as shown in Table 1.

Table 1: The Bacteria colony count

Sample	Sample size	Range count	Mean count	cfu/ml
Yellow	100	320	125.8	126 x 10^6
White	100	268	112.6	1.13 x 10^6
Chocolate	100	4	8.25	8.25 x 10^4

Table 2: No of positive and negative sample with their percentages to bacterial contamination.

Sample	No. positive	No. negatives	% Positive	% Negative
Yellow	70	30	70	30
Chocolate	60	40	60	40
White	100	00	100	00

Table 3: Bacteria Isolated in each sampled bread.

Bacteria	Bread Sample			
	Yellow	Chocolate	White	Frequency
Staphylococcus saprophyticcus	+	-	-	2
Staphylococcus aureus	+	-	+	3
Bacillus alvei	-	-	+	4
B. subtilis	+	+	+	5
B. licheniformis	+	-	+	4
Coryenbacterium sp	-	+	+	3
Proteus sp	+	-	+	2

Key + = Isolated - + non Isolated

Table 4: Fungi Isolated in the samples.

Yellow	Chocolate	White
Aspergillus niger	*Aspergillus niger*	*Aspergillus niger*
Mucor mich	*Mucor mich*	*A. oryzae*
Rhizopus stolonifer	*Rhizopus stolonifer*	*A. Flavus*
Penicillium sp.	*Penicillium sp.*	*Mucor mich*
		R. Stolonifer
		Penicillium sp.

Discussion

Food borne diseases are as a result of consumption of contaminated food by microorganism. *Staphylococcus saprophyticus* was isolated in yellow bread but not in white bread, whereas *S.aureus* was isolated in both. The presence of *S.saprophyticus* which is a normal flora of nose, hand and skin of animal and humans (6) should not be alarming, however, the presence of *S.aureus* in the two types of bread,

Yellow and white is of concern. *S. aureus* is known to be the cause of staphylococcal food poisoning by virtue of the preformed enterotoxin it produces in food. The poisoning is as a result of enterotoxin B in such food. Also incriminated are *Bacillus* organisms. *B. Subtilis, B.lincheniformis. B.subtilis* was isolated from the three bread samples, while *B.licheniforms* was isolated from the Yellow and white bread. The heating and the high baking temperature could not destroy the spore of the organisms.

Bacillus species are widely distributed in nature, mostly as saprophytes in the soil, dust, water and on vegetation where they form resistant spores (6) *Bacillus subtilis* produces toxins and also incriminated as causes of food poisoning (7). Other Bacilli organism isolated are *Bacillus licheniformis,* and *B. alvei* which produce antimicrobial substances, enzymes, and pigments (8).

The presence of *B. alvei* on bread in this investigation is similar to those reported by Gilbert (9) from bread, vegetables and in water.

Proteus sp was isolated from yellow bread, while *Corynebacterium* sp was isolated from chocolate and white bread. These organism are inhabitants of the gastrointestinal tract.

The low bacterial count observed in the yellow and chocolate bread compared to white bread may be due to the colouring agents in them, these agents might equally have certain deleterious effect on the consumer. Thus, work should be done to determine the effect of these agents in human.

This investigations is of public health importance, it's therefore necessary that our bakers, the retailers as well as consumers should maintain good environmental sanitation during baking and handling processes. Though strains of *Proteus*cause encentric infection in human, proof of transmission by food is inconclusive (10).

Fungi isolated from the three samples are *Aspergillus niger; Mucor mich Rhizopus stolonifer, Penicillium sp,* in addition to these, *Aspergilus oryzae, Aspergilus flavus* and *Rhizopus stolonifer* were isolated in the white bread. *A flavus* is known to produce aflatoxin which is implicated in human illness (11).

The low bacterial count and occurrence obtained in the Yellow and chocolate bread compared to white bread suggests that the colouring agents used for the coloured bread might have some antibacterial activity therefore limiting the growth of microorganism in the coloured bread than the white bread. According to Bryan (10) the range at which contamination of food may occur due to bacterial food poisoning is $10^4 – 10^8$ cfu/g of a food, this number may matter much when toxin production is concerned. The minimum dose enterotoxins that causes food poisoning is yet to be determined, however Gilbert (9) consider that as little as 0.5ug/g of food may be sufficient enough.

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