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Assessment of Microbial Quality of Yoghurt Sold in Owerri Metropolis, Imo State

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Abstract
The assessment of microbial quality of yoghurt sold in Owerri metropolis was determined. Eight brands of yoghurt designated V-fa,D-fa,V-favi,D-favi,V-sy,D-sy,V-Dsa and D-Dsa were obtained from Owerri metropolis. A tenfold serial dilution was carried out and appropriate diluted samples inoculated on duplicate plates of SDA, Nutrient agar, SSA and CLED agar. Discrete colonies that developed were counted, purified and stored on agar slant at 4^0C. for further identifications. The pH of the samples ranges from 5.60 to 6.0. The total coliform count ranges from 1.0 x10^4 cfu/ml to 1.2 x10^5 cfu/ml. The total viable bacterial count was 1.2 x 10^5 cfu/ml to 2.7 x 10^8 cfu/ml and fungal counts were 1.0 x 10^2 cfu /ml to 4.0 x 10^2 cfu/ml respectively. Three fungal genera were obtained as Aspergillus, Penicillium and Fusarium species. The bacteria isolates obtained include species of Staphylococcus, Lactobacillus, Escherichia, Bacillus and Klebsiella. The *Escherichia coli* occurred in all the yoghurt samples, *Klebsiella spp* and *Staphylococcus aureus* occurred in five yoghurt samples whereas Lactobacillus spp occurred in four yoghurt samples. The investigation clearly signifies that the microbes isolated are responsible for the microbial contamination of yoghurt. The microbial quality of yoghurt can only be improved through proper sanitary measures and excellent good manufacturing process.
Keywords*: microbial quality, yoghurt, Owerri*

Introduction
Yoghurt is a cultural dairy product produced by a lactic acid fermentation of milk using a combination of bacteria such as lactobacillus bulgaricus and streptococcus thermophilus in the ratio 1:1[1] Milk from which yoghurt is made is an excellent source of protein, vitamins and minerals like calcium and some antibacterial substances such as lysozyme and lacto peroxidase, as well as large amount of lactose sugar, peptone, phosphate and nitrogen-based enzymes.[2] Yoghurt has been described as a nutritiously balanced food containing almost all the nutrients balanced food containing almost all the nutrients present in milk but in a more assailable form.[3] Microorganisms

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present in fermented dairy products stabilize bowl microflora. Yoghurt is made from skimmed milk usually from cows, sometimes from other animals such as goat or sheep. Milk from which yogurt is made is an excellent source of protein, vitamins and minerals like calcium and some antibacterial substance such as lysozyme and lactoperoxidase, as well as large amount of lactose sugar, peptone, phosphate and nitrogen-based enzymes. The milk used in yoghurt production is homogenized, heated to a temperature of between 85 -100◦c and then cooled to 41-45◦c. The special starter cultures, streptococcus salvarius sub sp. Bulgaricus are then added. The yoghurt is Incubated for 3 hours at a temperature of 40◦c.

Further contamination of yoghurt by microorganisms can occur during milking, handling, storage and other processing processes, clearing and handling of milking utensils. Health complications associated with consumption of inadequately pasteurized milk products include serious infections that are hard to treat with antibiotics. This becomes clinically significant if organisms isolated from an assessed sample is resistant to conventional antibiotics. Thus, can confer antibiotic resistance to the infected host while providing no alternative drug.[4] Heat treated yoghurt do not contain lactic acid bacteria as these are killed during post fermentation. Yoghurt manufacturing companies mainly market "heat treat" yoghurt to prolong its shelf life.[5] It is important however to evaluate the microbial gravity of some milk products sold in Nigeria. The project work aims at assessing the antimicrobial susceptibility pattern of microorganisms present in yoghurt sold in Owerri metropolis.

Materials and Methods

Study Area

This study was carried out in Owerri municipal council of Imo state south east Nigeria between May to October 2023.

Sample Collection

Six different brands of sachet packaged beverage yoghurt were brought from hawkers and beverage stores in Owerri metropolis. One samples of each brand were used and the brands were designated A, B, C, D, E, F giving a total of 6 yoghurt samples. The samples were brought to the laboratory and analysis within 6hours of collection.

Sterilization of Material and Culture Media

The materials used for this study were sterilized by appropriate methods to free them from microbial contamination. All the media used were sterilized at 121^0c for 15 minutes in an autoclave and were prepared according to the manufacturer's instruction or standard methods for examination of water. Culture media generally use for the study are nutrient agar, CLED, SSA and sabour and dextrose agar (SDA). All glass was sterilized in the hot air oven at 160^0c for two hours. The inoculating needle and wire loop were sterilized by flaming in the Bunsen burner until red hot, working surface were sterilized by the application of disinfectant/antispectic solution (95% ethanol).

Preparation of Culture Media

Media used include:

SSA, CLED, chocolate agar and sabourand dextrose agar (SDA). They were prepared aseptically according to the manufacturer's instruction.

Microbial Analysis

Culture and Isolation

Bacteria Culture

1ml suspension of each yoghurt was sample pipette aseptically into 9mls of sterile water accordingly in tube 1 and the tubes were mixed thoroughly, with the sterile diluents, then further dilutions were made. 1ml of the appropriate dilution was plated in duplicate using a spread plate method on SSA, Nutrient agar, chocolate agar and CLED. The SSA, nutrient agar, chocolate agar plates were incubated at 37^0c for the 24 hours. The colours that developed on the chocolate agar, nutrient agar and CCED plates were counted and used to determine the total bacterial count of the yoghurt samples (cfu/ml). Representative colonies on the plates were sub-cultured on the fresh nutrient agar to obtain pure cultures of the isolates. the pure cultures were then transferred onto nutrient agar slants for biochemical identification.

Fungi Culture

1ml of serially diluted samples was inoculated into the Sabourand dextrose agar plated and slant which was mixed with 2% chloramphenicol and then incubated at room temperature (28^0c) for 5 days. the colonies that grew were counted and sub cultured on fresh Sabrourand dextrose agar plates to obtain pure cultures. They were later stored on SDA slants for characterization and identification.

Identification

Gram staining technique

Procedure

With the aid of a wire loop, the isolate was smeared onto a clean clear grease free slide and allowed to air dry. The slide was placed on a staining rack and was covered with the primary stain, crystal violet.it was allowed to stain for 60 seconds and was washed of gently with distilled water for 5 seconds. It smear was covered with Lugols iodine for 60 seconds and was rinsed in distilled water. It was decolourised rapidly with 95% alcohol, and was rinsed with distilled water. It was counter stained using neutral red for 60secs and was rinsed with distilled water and allowed to air dry. A

drop of immersion oil was placed on the stained slide and was viewed under the microscope using 100 x objective lens.

Catalase Test

Procedure

Few colonies of the organism were emulsified in distilled water on a clean slide placed in a petri dish. Two drops of hydrogen peroxide were added and mixed. A positive reaction was indicated by gas bubbles.

Coagulase Test

Procedure

A drop of distilled water was placed on a clean grease free slide. Colonies of the test organism (previously checked by the gram staining) was emulsified on the distilled water and a loopful of plasma was added, mixed gently and checked for clumping of the bacteria within 10 seconds. Clumping within 10 seconds indicates the presence of *S. Aureus*. this is a confirmatory test for *Staphylococcus aureus.*

Lactophenol cotton Blue Test for fungi

Two drops of lactophenol cotton blue reagent was pipette on a grease free clean slide. With the aid of a sterilized wire loop, the colony was collected and emulsified on the slide. It was incubated for 10 minutes on a cotton wool soaked with water placed into a petri dish. After incubation, it was viewed microscopically using x10 objective lens.

Flagella staining

(Loefflev's Silver method for flagella)

Procedure

A thin smear of the bacteria culture was made and fixed with gentle heat. The slide was flooded with loeffler's mordant for 5minutes. It was washed with distilled water. It was stained with heated loeffler's flagella stain for three minutes. It was washed with distilled water, dried and mounted. It was viewed under the microscope.

Citrate Utilization Test

Procedure

A light suspension of the organism was emulsified in saline Simmoms citrate agar was stab inoculated with a straight wire loop. A growth of blue colour was observed in Smmoms agar as a positive result. This meant that citrate has been utilized.

Motility Test

Procedure

A semi solid agar medium was prepared in a test tube. using a straight wire loop, motility medium was stab inoculated with the test organism.it was inoculated at 37^0c for 48 hours (Ochei and kolhatkar,2000).

Indole Test

Procedure

The test organism was inoculated in a bijou bottle containing 3ml of sterile tryptophan water.it was incubated at 37^0c for 48hours .0.5ml of Kolac" s reagent was added to the bottle, shook gently and examined for a red colour on the surface layer within 10 minutes.

Results

The data obtained on microbial count and PH determination of the yoghurt samples are shown in Table 1.the total aerobic count ranges from $1.2x10^5$ to 2.7×10^8 Cfu/ml. Among the samples, the total coliform counts ranged between 1.0×10^4 to 1.2×10^5 cfu/ml while the total fungal count ranged between $1.0x\ 10^2$ to 4.0×10^2 cfu/ml the PH was between 5.60 and 6.0.

Table 2 the bacteria isolates as presented were characterized based on morphology and biochemical identification as belonging to the genera staphylococcus, bacillus, lactobacillus, Escherichia and klebsiella. Yoghurt sample D. fa, V. fa, u-sy and D.sy had the highest number of bacteria isolated closely followed by sample V-fa and D-favi had same number of bacteria isolated. While V-Dsa and Dsa had low number of bacteria isolated. Escherichia coli was present in all the sample while staphylococcus, Bacillus, klebsiella and lactobacillus was present in the few samples.

Table 3 shows the fungal Isolates of the different yoghurt samples. They include species of Aspergillus, penicillum, and fusarium. Penicillum was present in sample D. fa, V-favi and D-favi, Fusarium was present in only two samples V. fa and V-Sy while Aspergillus specie was present in V-Fa, D-Sy, V-Dsa and D – Dsa samples.

Table 1: Microbial Counts and PH of Yoghurt Samples

Sample	PH	Total coliform count cfu/ml	Total bacterial count cfu/ml	Total fungal count cfu/ml
V.fa	5.60	$1.0x10^4$	$1.2x10^5$	$1.0x10^2$
D.fa	5.68	$1.1x10^4$	$1.3x10^6$	
	$1.0x10^2$			

V.favi	5.80 $1.1x10^2$	$1.2x10^4$	$1.4x10^8$
D.favi	5.82 $1.4x10^2$	$1.1x10^4$	$1.5x10^5$
V.sy	5.83 $4.0x10^2$	$1.6x10^4$	$1.7x10^5$
D.sy	5.86 $1.0x10^2$	$1.8x10^4$	$1.8x10^5$
V.Dsa	5.91 $2.0x10^2$	$1.9x10^4$	$2.6x10^6$
D.Dsa	6.00 $2.0x10^2$	$1.2x10^5$	$2.7x10^8$

Table 2

BIOCHEMICAL IDENTIFICATION

Organism Identified	Macroscopic Examination			Microscopic Examination						Biochemical Identification				
	Opacity	colour	Shape	Grame	Catalase	Indole	Oxidase	Mortility	Couglase	Citritate	Sporing	Capsutated	Mannitol	
Escherichia Coli	Opaque	Deeped	Rod	-	+	+	+	+	+	-	-	-	+	-
Staphylococcus aureus	Opaque	Golden Yellow	Cocci	+	+	+	-	-	+	+	-	+	+	-
Bacillus Spp	Opaque	Greyish white	Baccil	+	+	+	-	+	+	+	+	+	+	+
Klebsiella	Opaque	Cream	Rod	-	+	-	-	-		+	-	+	+	+
Lactobacillus	Opaque	White yellow	Rod	+	-	-	-	-	-	-	-	-	-	+

Table 3: Distribution of Fungal Isolate from the yoghurt samples

Fungal species	V. fa	D. fa	V.favi	D. favi	V.sy	D. SY	V. Dsa	D. Dsa		
Penicillium spp		_	+	+		+	_	_	_	_
Fusarium spp		+	_	_		_	+	_	_	_
Aspergillus niger		+	_	_		_	_	+	+	+

Key: +present
-Absent

TABLE 4: Description of morphological characteristics of fungal isolates.

FUNGI	MORPHOLOGICAL CHARACTERISTIC
Penicillium spp	Colonies are usually fast growing in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores. Microscopically, chains of singled –celled conidia are produced in basipetal succession from a specialized conidpgenons cell called a phialide.
Fusarium spp	The colour of the thallus varies from whitish to yellow, pink, red or purple shades. species of fusarium typically produce both macroconidia and microconidia from slender phialides.
Aspergillus niger	Relatively small and regular in size with septad hyphae powdery black in colour with dotted conidia, the phialides covers the entire surface thick walled, rough and darkly pigmented, the underside of the colony as buffy.

Discussion

Studies of the microbial contamination of yoghurt samples, showed unacceptable levels of both bacteria and fungi Escherichia coli, staphylococcus aureus, bacillus spp, klebsiella spp and lactobacillus spp as bacteria present while fungi isolated include *Asperigillus spp, Peniccillium spp* andF*usarium spp.* Some species of Aspergillus have been implicated in the secretion of aflatoxins which are carcinogenions to human when consumed. Escherichia coli was the most isolated due to easily distribution through contaminated fecal material from humans and warm-blooded animals, unpasteurized milk a cow's udder, improper handling or processing and packaging could lead to high rate of Escherichia coli which are potential agents of food poisonings.

The presence of staphylococcus species in the yoghurt samples could also be as a result of processing, handling and packaging since they are often found on the outer surface of the body. Government in many countries acknowledge a code of hygiene practice as an important tool in this aspect and is called the code needs to lay emphasis on hygiene from the start of production of the yoghurt to the point of sales, could be considered the principle of codex document.[6] In processing and preparation adequate measure should be taken to reduce or eliminate pathogens to an acceptable level, in order to prevent growth of pathogens, production of toxic chemicals and the introduction of physical pathogens, and to ensure that foods and drinks are not re-contaminated.

Conclusion

The yoghurt brands according to the result of the investigation clearly signify that they are not of the same microbial quality. Bacteria and fungi isolated from yoghurt samples in this study proved that microorganism is responsible for microbial contamination of yoghurt. These can be reduced only through proper sanitary measures and excellent good manufacturing process.

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