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Determination of Microbial and Physicochemical Qualities of Six Brands of Yoghurt Sold in Ogwashi-Uku Metropolis

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Abstract

Yoghurt is a fermented dairy product, rich in protein content and possesses a gel-like texture. This study was undertaken to assess consumer preferences for yoghurt and to compare the nutritional, sensory, microbial and physiochemical qualities of six brands of yoghurt. The sensory analysis for color, taste and consistency were carried out, and it was observed that product A has a normal taste, while product B - E possesses a sweet taste, while product F has a sour taste, possessing a custard-like consistency. The total cultural heterotrophic bacterial and fungal count was observed, and product D possess a higher bacteria count, while product A possess a higher fungal count (Cfu/mL). A sensitivity culture test was carried out on five different bacteria isolate using the following antibiotics agents: Amoxicillin (AMI), Streptomycin (S), Chloramphenicol (CH), Norfloxacin (N), ciprofloxacin (CPX), Erythromycin (E), Gentamycin (CN), Rifampicin (RD), Ampiclox (APX), and Levofloxacin (Lev). The result showed that *Bacillus coagulans* was resistance to all the antibiotics. The result of this study further indicated poor microbiological standards of commercial yoghurts sold in Ogwashi-uku market in Delta state at the time of this research. It is recommended that yoghurt stored without a refrigerator over one day should be consumed to avoid an outbreak of contamination which can be called food poisoning. Also, manufacturers should apply hygienic processes when producing yoghurt to avoid contamination. Finally, yoghurt manufacturers and vendors should avoid long exposure of yoghurt, also quality control (QC) measures including good manufacturing practices (GMPS) should be encouraged.

Keywords: *Yoghurt, microbial contamination, dairy products, Bacillus*

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Introduction

Food is an essential ingredient to sustain life which can be obtained from plant and animals. Milk is one of such foods which can be obtained from animal sources (Beshart, 1982; Akram *et al*., 2020), and it has been established as natural and nature's most complete food due to its endorsed nutrient (Protein, carbohydrates, minerals, vitamins) (Everett and McLeod, 2005; Egbuna and Dable-Tupas, 2020).

Yoghurt is a fermented often flavored semi-solid food made from milk. Its production involves the fermentation of the lactose content in milk giving rise to lactose acid, acetic acid, carbon dioxide (Co_2) acetaldehyde, diacetyl etc through the use of starter culture which contains *Streptococcus thermophillus* and *Lactobacillus bulgaricus* (Adolfsson *et al*., 2013; Palai *et al*., 2020). According to report, yoghurt has almost the same nutritional value as the basic milk product (Buttass, 1997).

The consumption and demand for yoghurt have increased worldwide (Nutraceuticals world, 2010, Palai *et al*., 2020). In Nigeria for example, yoghurt consumption has been on the increase during the last decade largely by residents of both urban cities and rural areas (Dublin-Green and Ibe, 2005). This increase has led to the establishment of small scale factories solely for the production of yoghurt in many cities (Nwamaka and Chile, 2010). The study examined the yoghurt samples sold within the confines of Ogwashi-Uku metropolis to check and ascertain the physiochemical and microbial qualities.

Materials and Methods

Collection of Samples

Six most common samples of yoghurt sold in Ogwashi-uku Metropolis, Delta State in Nigeria was bought from Ogwashi-uku market and taken to the laboratory for analysis to be carried out.

Physical analysis

Physical analysis such as colour, taste and consistency of the yoghurt product samples were observed

Microbiological Analysis

Determination of total cultural heterotrophic bacteria count (THBC)

Total heterotrophic bacterial counts for each water sample were enumerated using spread plate method as described by Willey *et al.* (2008). An aliquant (0.1 mL) of the dilution of 10^3 were aseptically transferred unto properly dried nutrition agar plates in duplicate, spread evenly using bent glass rod and bate at 37ºC for 24 b. After incubation, the bacterial colonies that grew on the plate were counted and an average taken. The colony forming unit for the THBC of water samples Were then calculated usipo the formula; THFC (CFU/g) = Number of Colonies x Dilution factor 10) x volume plated (0.1 mL).

Determination of Total Cultural Heterotrophic Fungal Count

The total fungi in each of the water sample were enumerated using spread plate method as described by Willey *et al.* (2008). Aliquot of 0.1 mL from dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (tetracycline) to inhibit bacterial growth, in duplicate, spread evenly using bent glass rod and incubated at 28ºC for 3 days.

The fungal isolates which developed were counted and sub-cultured unto Sabouraud Dextrose.

Agar slant in Biou bottles for preservation and identification. Total heterotrophic fungal (THF) counts for each sample were calculated using the below formula: THFC (CFU/g) = Number of colonies/ Dilution factor (10) x Volume plated (0.1 mL).

Antibiotics Sensitivity Test

The Clinical and Laboratory standard Institute (CLSI) disc diffusion method was used for the antibiotic sensitivity test. The turbidity of the inocula of various isolates was made to be equivalent to 0.5 of McFarland standard and each of the isolates was inoculated onto the surface of Muller Hinton agar using sterile swab sticks. The antimicrob1al agents tested were: Ciprofloxaxin 10µg, norfloxacin 10µg, gentamycin 10µg, tarivid 10µg, reflacine 10µg ceporex 10µg, amoxicillin 20µg, rifampicin 20µg, ampiclox 20µg levofloxacin 20µg, erythromycin 20µg, streptomycin 30µg chloramphenicol 30µg, augmentin 30µg, nalidixic acid, septrin 30µg. ampicillin 30µg (Opton Disc, Nigerna). These were aseptically placed on the surface of the inoculated agar plates. After 30mins of applying the discs, the agar plates were inverted and incubated for 24 hrs at room temperature (Uba *et al*., 2018) clear zones that developed around each disc were measured as the zones of inhibition on the basis of CLSI guidelines.

Bacterial Identification

The five bacterial isolates were Gram Stained and their arrangement were considered. Also, biochemical tests such as motility, catalase, Oxidase, citrate, indole, urease hydrogen sulphide production, glucose, fructose and sucrose were done. The isolates were characterized and identified using Bergey's Manual of Determinative Bacteriology after the taxonomic studies were carried out (Uba *et al*., 2018).

Fungal Identification

The isolates were identified using the most standard and typical keys in fungal identification by comparing their colonial and microscopic description with those known taxa (Okoye *et al*., 2020).

Results and Discussion

In this study, physical analysis was carried out on the six samples gotten (Table 1). It was observed that product A is whitish in colour, tastes normal and is thick, product B is also white, sweet and very watery, product C is milky sweet and thicker, product D has a pink colour, sweet and watery, product E is white, sweet and watery, and product F is white, sour and has a custard-like consistency.

Table 1: Physical qualities of the different brands of yoghurt

Sample	Colour	Taste	Consistency
Product A	White	Normal	Thick
Product B	White	Sweet	Very watery
Product C	Milky	Sweet	Thicker
Product D	Pink	Sweet	Watery
Product E	White	Sweet	Watery

The total cultural heterotrophic bacterial and fungal counts in the yoghurts, data obtained show that product D has more bacteria count (5.07) and product A has more fungal count as displayed in Table 2.

Table 2: Microbial quality of different brands of yoghurt

Sample	Bacterial count (cfu/ml x 10^6) fungal count	Fungal count (cfu/ml x 10^6)
Product A	2.79	3.50
Product B	3.78	2.00
Product C	3.11	2.40
Product D	5.09	1.60
Product E	3,80	1.00
Product A	2.11	1.30

N/B: cfu/ml = colony forming unit millilitre

In the case of bacteria isolates pattern, a media was prepared using water, nutrient agar and a petri dish (plate), the agar was allowed to dry properly and the susceptibility pattern of bacterial isolates in response to different antibacterial agents (which include: Amoxicillin, Streptomycin, Chloramphenicol, Norfloxacin, Ciprofloxacin, Erythromycin Gentamycin, Rifampicin, Ampiclox, Levotloxacin) was shown in table 3.

Table 3: Susceptibility pattern of bacterial isolates

Abs	Isolates 1	Isolates 2	Isolate 3	Isolate 4	Isolate 5
Aml	0.00	1.70	0.00	0.00	1.60
S	0.00	0.00	0.00	0.00	1.40
NB	0.00	0.00	0.00	0.00	1.50
CH	0.00	0.00	0.00	0.00	1.50
Cpx	0.00	1.60	0.00	1.70	1.50
E	0.00	0.00	0.00	1.90	1.60
LEV	0.00	1.30	0.00	1.60	1.70
CN	0.00	0.00	0.00	1.30	1.50
APX	0.00	0.00	0.00	0.00	1.60
RD	0.00	1.40	1.40	1.20	1.20

N/B: Aml = Amoxicillin 20µg, S = streptomycin 30µg.
CH = Chloramphenicol 30µg. NB-Norfloxacin 10µg,
CPx = Ciprofloxacin 10µg, E = ervthromycin 30µg
CN = Gentamycin 10µg, RD = R. fampicin 20µg,
APx = Ampiclox 20µg, LEV = levofloxin 20µg

However, table 4 displays the susceptibility patterns of fungal 1solates, in response to different antifungal agents. The anti-fungal agent used are ketaconazole, Griseofulvin and Nystatin.

Table 4: Susceptibility pattern of fungal isolates

Zone of inhibition (mm)			
Isolates	**Ketoconazole**	**Griseofulvin**	**Nystatin**
1	2.20	0.00	1.30
2	1.70	0.00	1.60
3	0.00	0.00	1.20
4	0.00	0.00	1.70
5	1.70	0.00	2.20

Other isolated bacterial contaminants are shown in table 5. However, the contamination of all the yoghurt samples used for this research purpose could be either as a result of post-production contamination or poor health condition of the mammal (animals) whose milk was used. Table 6 shows the morphology of the fungal isolates.

Conclusion

This study revealed the health benefits alongside the nutritional value of yoghurts. However, the bacterial count in product D and fungal count in product A is perturbing and can compromise the aforementioned health and nutritional benefits, therefore proper hygiene and post-production processes should be maintained to avoid the introduction of microbes (contaminants) into the yoghurt products to curb the associated health implications/danger of poorly produced or contaminated yoghurt to the public.

Recommendations

1. Based on the analysis of the various yoghurt samples, it is recommended that yoghurt stored without refrigeration over one day should be consumed to avoid outbreak of contamination which can also be called in other word food poisoning.
2. Manufacturers should apply better hygienic process when producing yoghurt to avoid contamination.
3. Finally, yoghurt producers/manufacturers, vendors and handlers including consumers should avoid long exposure of yoghurt, also quality control (QC) measures including Good manufacturing practices (GMPS) should be encouraged.

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Table 5: Biochemical profile of bacterial isolates

Parameter	**Isolates**				
Identity	1 *Bacillus coagulans*	2 *Citrobacterial specie*	3 *Klebiellaoxytoca*	4 *Micrococcus specie*	5 *Proteins mirabilis*
Gram reaction	+ ve Rod	-ve Rod	-ve Rod	-Ve cocci	- ve Rod
Oxidase	+	+	+	-	-
Catalase	+	+	+	+	+
Urease	+	-	-	-	+
Indole	-	+	+	+	-
H_2S	+	-	-	-	-
Motility	-	+	+	-	+
Starch hydrolysis	+	+	+	+	+
Citrate	+	+	+	+	+
Sucrose	+	+	+	+	+
Glucose	-	-	+	+	+
Fructose	+	+	-	+	+

NB: +Ve= positive, -Ve =Negative

Table 6: Morphological features of fungal isolate

Isolate	**Cultural feature**	**Microscopic feature**	**Identity**
1	Possess bluish green surface with entire margin irregular and flat form	Non-septate brush arrangement of phialospores	*Penicillium citrum*
2	The colonial margin is entire with a flat elevation, irregular form, white in colour and absent of aerial hyphae	Produced a septate hyphae with coridia mass containing ascospores	*Hypocrea sp*
3	Filiform colonial margin, raise in elevation with whitish filamentous form	Possess large number of tiny septate ascospores	*Fusarium sp*
4	Possess deeply cottony texture of white to gra-brown surface	Possess a non-septate sporangiospores	*Rhizopus sp*
5	Produced gray to brownish gray appressed mycelia in segmentation	Produced ascospores from perithecia and appresoria	*Hhysalospora vacani*

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