

Assessment of Mycological Quality of Commercially Sold Yogurt in Uturu, Abia State, Nigeria

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Abstract

Assessment of the mycological quality of commercially sold yoghurt in Uturu, Abia State, Nigeria was carried out in this study. Five different brands of yoghurt coded A to E were analyzed using standard microbiological methods. Total fungal count ranged from 0.5×10^1 cfu/ml – 0.6×10^3 CFU/mL. Fungi isolated include *Aspergillus* sp., *Penicillium* sp. And *Rhizopus* sp. Antifungal susceptibility studies showed susceptibility of fungal isolates to all antifungal agents tested including Amphotericin-B (20 µg), Itraconazole (10 µg), fluconazole (25 µg) and Ketoconazole (10 µg). The presence of fungi in the yoghurt may be as a result of unclean water used in the production, contaminated milk and unhygienic condition of the handlers. It is recommended that yoghurt producers, sellers and handlers should ensure hygienic practices and avoid long exposure of yoghurt before selling to consumers.

Keywords: Yoghurt, Fungi, Anti-fungal agents, Uturu, Abia

INTRODUCTION

Yoghurt is a product of milk coagulation resulting from the fermentation of lactose in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (Adolfsson *et al.*, 2004). Other lactic acid bacteria (LAB) are also frequently used to produce yoghurt with a unique characteristic (Adolfsson *et al.*, 2004).

Yoghurt is commercially produced by pasteurizing milk and allowing it to cool to 45°C before been inoculated with starter culture. The starter culture is usually a mixed culture of *Streptococcus thermophiles* and *Lactobacillus bulgaricus* in a ratio of 1:1 (Obukokwo *et al.*, 2017).

They act on lactose and result in the production of lactic acid which increases the acidity of the yoghurt, thereby forming gel (Obukokwo *et al.*, 2017).

Yoghurt is an easily digested product of milk; this is because in milk, protein, fat and lactose components undergo partial hydrolysis during fermentation (Oluwafemi and Da Silva, 2006). Yoghurt is produced by microbial synthesis; therefore species of bacterial, yeast and mould different from those normally found in active yoghurt cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) are present in plain yoghurt (Oluwafemi and Da Silva, 2006).

Yoghurt has been ranked as the most available fermented milk product in the world today. Its mildly acid flavour, custard texture coupled with nutritive value made it to be among the commonly relished foods throughout the history of mankind (Taura *et al.*, 2005).

Production of yoghurt is a classic example of process employing the beneficial role of some microorganisms like *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1 to serve as the starter culture for the transformation of fresh milk to yoghurt under suitable physico-chemical conditions (Obiekezie *et al.*, 2012). Aside that, such products are not expected to contain undesirable microorganisms beyond certain tolerable limits (Taura *et al.*, 2005). Spoilage by bacteria and moulds is possible due to unclean equipment, poor hygiene of the production staff, contaminated milk and not maintaining the correct incubation temperature (Oluwafemi and Da Silva, 2006). Yoghurt is usually packaged and sold in disposable paper packs, polythene bags and or in plastic bottles (Dublin-Green and Ibe, 2005). Final packaging which includes sealing of the content of such drinks has been reported to be usually done by hand or by use of simple mechanical sealing machine in small and medium scale factories as opposed to the use of automated filler. Yoghurt should be stored at chilling temperature not higher than 5 °C for a longer shelf life. On the contrary, they are usually held in storage compartments at about 10 °C or left in open shelves in some supermarkets and are even sold by hawkers carrying the yoghurts in open cartons exposed to the heat of the sun. Yoghurts not properly refrigerated are prone to spoilage by yeast contaminants (Dublin-Green and Ibe, 2005). The quality of yoghurt in the market varies from one producer to another. A practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in the market for microbial contaminants (Obiekezie *et al.*, 2012).

MATERIALS AND METHODS

Study Area

Uturu is a town located within latitudes 05.33°N and 06.03°N, in the northern part of Abia State, Nigeria. It is in the transition from rural to urban status, so it is witnessing many development activities (Chigbu, 2015). It is popularly known as a location for several educational institutions and the Marist Brothers community. Schools in Uturu include Abia State University, Marist Brothers' Juniorate, Uturu, Gregory University, and several post-secondary schools.

Sample Collection

Five samples were selected and collected for this study. Four of the samples were sealed and contained in plastic bottles. One sample was contained in a sachet. All samples were collected at

the point of sale and ready for consumption. All the yoghurt tested were duly registered by the National Food Drug Administration and Control (NAFDAC).

Selected yoghurt samples were bought from different vendors within Uturu community, Isukwuato Local Government, Abia State. The 5 samples were coded as A-E. Coded samples were transported in ice packs and eventually analyzed for mycological quality at the Advanced Microbiology Laboratory of the Gregory University, Uturu.

Media Preparation

All the media used were prepared according to the manufacturers' Instructions.

Mycological Studies

The determination of mycological loads in the yoghurt samples were carried out. Sabouraud Dextrose Agar was used for fungal count. The media were sterilized in an autoclave at 121 °C for 15 minutes. Diluents were prepared aseptically by pipetting 9 mL of distilled water into 5 tubes and sterilized at 121 °C for 15 minutes in an autoclave. 1 mL of the labelled yoghurt sample was introduced into the cooled sterile water and serial dilution was done to 10⁻⁵ dilution factor. 1 mL of the appropriate diluted sample was transferred into freshly prepared Sabouraud Dextrose Agar that has been sterilized and cooled to about 45 °C. The plates were gently swirled clockwise and anticlockwise for even distribution of the available fungi, allowed to solidify and incubated at an inverted position at room temperature for 24 - 48 hours. It was removed and observed for colonies (Willey *et al.*, 2008). Observation recorded and result expressed as colony forming units (CFU) (Oluwafemi and Da Silva, 2006). These analyses were done within the interval of 2 days with each yoghurt sample.

Total Fungal Count

For enumeration of the total fungal count, 1 mL of each sample dilution was seeded on previously prepared Sabouraud Dextrose Agar (SDA) plates using pour plate method. The plates were then incubated at 25±2°C (room temperature) for 24 - 48 hours. The fungal colonies were counted and expressed as log₁₀ colony forming unit per milliliter (log₁₀ CFU/mL) of the yoghurt sample.

Purification and Maintenance of Pure Cultures

After 24-48hrs of incubation, the different discrete colonies of fungi were counted and saved on SDA slants for further analysis.

Identification of Fungal Isolates

A speck of fungal growth from Sabouraud Dextrose Agar (SDA) plate was teased out using a sterilized straight wire and placed on a grease free microscope slide. A drop of lacto phenol cotton blue stain was added and covered with a cover slip. The mycelium was then examined using the light microscope at x40 objective (Cheesbrough, 2003).

Determination of Activity of Different Antifungal Agents against the Fungal Isolates

Isolates from the yogurt samples were tested for susceptibility to standard conventional antifungal agents like, Amphotericin-B (20µg), Itraconazole (10µg), fluconazole (25µg), Ketoconazole (10µg) manufactured by Abtek Biological Ltd, England. A loop-full of fungal growth of each isolate on nutrient agar slant was suspended in sterile normal saline. The turbidity was adjusted to the equivalent to 0.5 MacFarland standard that is a density of 1x10⁸

cells/mL before inoculation. Sabouraud Dextrose Agar plates were inoculated with 0.5 mL of fungal suspension adjusted to 1×10^8 cell/mL Using forceps sterilized over the Bunsen flame, sensitivity disc containing antifungal agents were placed on the surface of each agar plate evenly seeded with test isolates and was incubated for 24 - 48 hours at 25 °C (Cheesbrough, 2003).

RESULTS

Table 1 shows the enumeration of fungal isolates obtained from commercial yoghurt samples in Uturu, Abia State. The total fungal count for samples A, B, C, D, E are 0.6×10^3 CFU/mL, 2.0×10^1 CFU/mL, 0.8×10^1 CFU/mL, 0.5×10^1 CFU/mL and 1.0×10^2 CFU/mL respectively.

Identification of the fungal isolates based on cultural and microscopic characteristics showed the presence of *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp. (Table 2)

Susceptibility of fungal isolates to antifungal agents such as Amphotericin-B (20µg), Itraconazole (10µg), fluconazole (25µg), Ketoconazole (10µg) was carried out. Analyses showed that the fungal isolates were susceptible to all the antifungal agents used in this study (Table 3).

Table 1. Enumeration of Fungal Isolates

	Samples				
	A	B	C	D	E
TFC(CFU/mL)	0.6×10^3	2.0×10^1	0.8×10^1	0.5×10^1	1.0×10^2

Key: TFC = total fungal count, A, B, C, D, E, = sample codes

Table 2: Characteristics of fungi isolated from yogurt samples sold in Uturu, Abia State

Colonial Characteristics				Microscopic characteristics	
Nature of Colony	Reverse Side	Texture	Nature of Growth	Characteristics	Organism
Wooly, Whitish yellow edge with a covering of brown which then turns dark brown to black	White to yellow	Wooly	Rapid	Septate hyphae made up of unbranched conidiophores arising from specialized foot cell. Tip of conidiospore is enlarged forming a rounded vesicle which are covered with flask-shaped chains of smooth dark brown conidia.	<i>Aspergillus</i> spp
Blackish fluffy colouration with powdery appearance	Black	-	Slow	Pale brown slightly curved Conidia	<i>Rhizopus</i> spp.
Pale green appearance at the centre Velvety and rough walled	Yellowish		Raid	Map-like conidiospores, Septate hyphae and Globose	<i>Penicillium</i> spp.

Table 3: Antifungal susceptibility pattern of fungi isolated from yogurt samples sold in Uturu, Abia State (mm)

	Amphotericin-B (20µg)	Itraconazole (10µg)	fluconazole (25µg)	Ketoconazole (10µg)
<i>Penicillium</i> spp.	16.0±0.6	20.0±0.2	15.2±0.1	15.0±0.3
<i>Aspergillus</i> spp	17.8±0.5	19.5±0.1	10.3±0.3	12.0±0.1
<i>Rhizopus</i> spp.	22.0±0.1	26.4±0.5	28.0±0.1	18.5±0.5

Interpretative standard: Clinical and Laboratory Standard Institute (CLSI) (2006).

DISCUSSION

The fungal isolate identified in this study were *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. Obiekezie *et al.* (2012), also reported similar organisms in yogurt (a locally brewed yoghurt). The source of these microorganisms may be from air as moulds are common air contaminants. Some species of the *Aspergillus* genus have been reported to produce aflatoxin which causes lung diseases, aspergillosis and otomycosis (Tanaka *et al.*, 2023). Similarly, most *Aspergillus* species are human and livestock pathogens associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic and naso-orbital infections (Dunne *et al.*, 2023). The presence of *Aspergillus* and *Rhizopus* species in this study agreed with Ifeanyi *et al.* (2013) who reported that moulds are primary contaminants in yoghurt produced in Nigeria. The presence of these moulds in the yoghurt samples was expected considering the low level of sanitation, hygiene and development in Uturu Community.

The fungal isolates were all susceptible to the antifungal agents such as Itraconazole with an inhibition zone values ranging from 19.5±0.1- 26.4±0.5 mm. Itraconazole is a triazole with activity against a large number of fungi including yeasts, as well as dermatophytes, *Aspergillus* species and dimorphic fungi. Ketoconazole is an imidazole which is used orally, requiring systemic absorption which depends on the gastric acidity. Since ketoconazole can be administered orally and shows a wide antifungal spectrum, the drug is a natural candidate for use in the systemic treatment of keratomycosis, either alone or in association with topic preparations (Jodh *et al.*, 2023). In this study, the fungal isolates were susceptible to ketoconazole with an inhibition zone values. Fluconazole is a triazole which exhibits excellent tolerability and intraocular penetration.

CONCLUSION

In conclusion, fungi isolated from the commercially sold yoghurt in Uturu included *Penicillium* sp, *Aspergillus* sp and *Rhizopus* sp. The fungal isolates were all susceptible to the antifungal agents used, these include Amphotericin-B, Itraconazole, fluconazole and Ketoconazole. The presence of fungi in the yoghurt may be as a result of unclean water used in the production, contaminated milk and unhygienic condition of the handlers. Based on the analysis of the various yoghurt samples, it could be recommended that yoghurt producers, sellers and handlers should avoid long exposure of yoghurt before selling to consumers. Quality Control (QC) measures with Good Manufacturing Practices (GMPs) should be encouraged. Similarly, standard organization for control of industrial products should ensure that strict measures are taken for compliance to standard.

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