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MICROBIOLOGICAL EXAMINATION OF COMMERCIAL PROBIOTIC YOGHURT PRODUCED AND SOLD IN MAKURDI, BENUE STATE, NIGERIA.

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ABSTRACT: Studies were carried out to ascertain the presence of pathogens in commercial probiotic yoghurt produced sold in Makurdi. This is with a view to ascertaining their hygienic conditions. Two batches of commercial probiotic yoghurt were examined (Batch A and Batch B). Batch A was examined after 49 days of storage (11 days to the expiry date) and Batch B was examined after 11 days of production (49 days to the expiry date). Microbiological examination shows the presence of *Staphylococcus aureus* and *Mucor spp* in Batch A, and *Bacillus spp* in Batch B. This result implies that Batch A expired before the expiry date on the labels. For Batch B, the identification tests were not sufficient to reach definite conclusions but the result may also suggest contamination due to handling in the production process. The presence of these pathogenic organisms suggests grave health implications for the consumers who rely on the expiry dates on the labels. The implications of these findings to health generally and the regulatory processes are discussed.

KEY WORDS: Probiotic yoghurt, Pathogenic organisms, Hygienic conditions, Microbiological, Examination, Makurdi, Nigeria.

INTRODUCTION

Traditionally, yoghurt is manufactured using *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* as starter cultures (Shah, 2000). For the production of probiotic yoghurt, probiotic bacteria, usually *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are incorporated into the starter culture as dietary adjuncts (Shah, 2000). Probiotic yoghurt is produced by the marriage of these beneficial bacteria to yoghurt. The fermentation process in probiotic yoghurt produces billions of friendly bacteria that are antagonistic to a wide range of pathogens especially in the gut (Ejembi, 2007).

A good probiotic yoghurt should meet the following criteria (i) it should not be pasteurized after production ii) it should be stored below 10^0C always to ensure the survival and viability of the probiotics and iii) the P^H of the yoghurt should be 4.5 or less (Shah, 2000). Failure to meet these criteria may pave way for pathogenic organisms to contaminate the yoghurt. Hence, any pathogen that contaminates the yoghurt due to the above reasons or poor hygiene practice during production and packaging will also be preserved in the yoghurt, thereby posing a health hazard to consumers.

Yoghurt is a favourite drink to many Nigerians. In Makurdi, the consumption of yoghurt has increased over the last three years mainly due to the introduction of probiotic yoghurt with its acclaimed health benefits. However, these products have not been subjected to serious microbiological examination to ensure their sanitary conditions. The occurrence of pathogenic bacteria in soured milk and the growth and metabolite production by *Penicillium brevicompactum* in yoghurt has been reported in recent researches in Zimbabwe and Italy, respectively (Gran *et al*., 2003 and Ndagijimana *et al*., 2008). Pathogenic organisms have also been isolated from commercially produced yoghurt in Lagos, Nigeria (Green *et al*., 1987). The high number of *Staphylococcus aureus*, *Escherichia coli* and other pathogenic organisms in yoghurt represent a health risk to consumers and emphasize the need for increased hygiene practice in the yoghurt industry.

The primary contaminants in yoghurt are yeast and molds. This is mainly due to the ability of these organisms to tolerate the high acidic condition of yoghurt (Pederson, 1979). The presence of yeast in yoghurt gives it an alcoholic taste whereas the contamination of yoghurt by molds results in the production of mycotoxin (Pederson, 1979). Green and Ibe (1987) reported that these organisms have been isolated from commercially produced yoghurt in Lagos, Nigeria.

The most widely encountered mycotoxin in foods contaminated by molds are aflatoxins which are the metabolic products of *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin causes serious diseases in man and Carcinogenesis in other animals (Edds, 1973).

Foschino *et al*. (1993) discovered in Italy while microbiologically examining brands of yoghurt (A, B and C) that in A samples, *Acetobacter aceti* was found at 10^7cfu/g, while in B and C samples the main contaminants were *Mucor hiemalis*, *Mucor racemosus* and *penicillium verrucosum* var *cyclopium* varying between 10^2cfu/g and 10^5cfu/g.

All above literature indicates that no work has been done in this area in Makurdi. This work is therefore aimed at ascertaining the microbiologic quality of commercial probiotic yoghurt produced in Makurdi metropolis, Benue State of Nigeria.

MATERIALS AND METHODS

The laboratory work was carried out in the Advanced Microbiology Laboratory of the University of Agriculture, Makurdi, Nigeria.

Collection of samples

The probiotic yoghurt samples were bought directly from the head office of the dairies producers in Makurdi. The samples were randomly selected from two batches-Batch A (sample 1) and Batch B (sample 2). Batch A was stored in the refrigerator for 49 days (11 days to the expiry date) before examination while Batch B was examined after 11 days of production (49 days to the expiry date).

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Inoculation/isolation

10^{-2}ml dilution factor of the probiotic yoghurt from Batch A was prepared and inoculated into nutrient agar by the spread plate method and incubated for 48hrs at 37^0C under aerobic condition. The growth on Nutrient agar was sub-cultured into CLED, PDA and MacConkey agar. The sub-cultured plates were also incubated at 37^0C under aerobic condition for 48hrs.

Serial dilution of the probiotic yoghurt from Batches A and B ranging from 10^{-1} to 10^{-3} was prepared. They were then inoculated directly into Nutrient, PDA, MacConkey and CLED agar by the spread plate technique. NA, MA and CLED agar plates were incubated at 37^0C for 48hrs under aerobic condition while PDA plates were incubated at room temperature for 72hrs.

Identification of Fungi

The identification of fungi was based on spore-formation and the morphology of spores. Colour, texture, form and rate of growth were also used in the identification.

For microscopic examination, slides were prepared by smearing distinct colonies of the specimens in droplets of ethanol on microscopic slides and then staining with Lactophenol blue solution. The identification tests were based on the procedure outlined by Cheesbrough (2000).

Identification of bacteria

The identification of bacteria was based on their Gram staining reaction, Cultural, morphological and biochemical characteristics using guidelines from Cheesbrough (2000).The following biochemical tests were further performed on the specimens:

Catalase test

With a wire loop, a colony sample from each culture was introduced into a clean test tube. Few drops of Hydrogen peroxide (H_20_2) were then added to the sample. Presence of gas bubbles indicates that the organism is catalase positive while absence of bubbles indicates that the organism is catalase negative (Cheesbrough, 2000).

Coagulase test

The test organism was picked from a discrete colony and placed in a few drops of water on a microscopic slide. Few drops of plasma was added to the sample and rocked gently. Coagulation of the sample indicates that the organism is coagulase positive.

RESULTS

A total viable count of $8.2x10^3$ cfu/ml was counted on Nutrient agar after incubation for 48hrs under aerobic condition. The characteristics from the growth gotten from sub-culturing on CLED, PDA and MacConkey agar is shown in table 1.

The characteristics of the growth on CLED, Nutrient agar, MacConkey and PDA from sample 2 (Batch B) are outlined in table 2.

Table 1: Identification of growth from sample 1(Batch A) on CLED, MacConkey and Potatoes Dextrose Agar.

Growth of organisms on	Colony/Microscopy Characteristics	Gram reaction	Catalase	Coagulase	Suspected organism
CLED	Spherical cells arranged in irregular clusters with smooth, raised and glistening colonies. Colonies are grey to deep golden yellow in colour.	Positive	Positive	Positive	*S. aureus*
MacConkey	Colonies are punctiform in shape. Appears singly and in clusters	Negative	Positive	Negative	Ni
PDA	Fills the petridish within 3 days with a grey white, fluffing and cottony mycellium. Hyphae are aseptate, broad, ribbon-like and twisted.	Dark purple	ND	ND	*Mucor spp*

Key:
NI = Not identified ND = Not done

Table 1 shows the isolates from Sample 1 (Batch A). The isolate from the growth on CLED agar was identified as *Staphylococcus aureus*. *Mucor spp* was isolated from the growth on PDA. However, the growth on MacConkey agar could not be identified.

Table 2 shows the isolates from Sample 2 (Batch B). No visible colony was observed on MacConkey, Nutrient agar, and PDA after 72hrs of incubation. However, *Bacillus spp* was isolated from the growth on CLED after 48hrs of incubation under aerobic condition.

Table 2: Identification of growth from sample 2 (Batch B) on CLED, MacConkey, Nutrient and Potatoes Dextrose Agar

Growth of organisms on	Colony/Microscopy characteristics	Gram reaction	Catalase	Coagulase	Suspected organism
CLED	Light green, smooth, flat and translucent colonies.	Positive	Positive	Negative	*Bacillus spp*
MacConkey	Nil	ND	ND	ND	No visible colonies
NA	Nil	ND	ND	ND	No visible colonies
PDA	Nil	ND	ND	ND	No visible colonies

Key: ND = Not done NA = Nutrient agar

DISCUSSION

Staphylococcus aureus and *Mucor spp* were isolated from Sample 1(Batch A) from the growth on CLED and PDA respectively after 72hrs of incubation under aerobic condition at 37^0C. However, the growth on MacConkey agar could not be identified

The result is in agreement with the works of Umoh *et al*. (1985) and Park *et al.* (1992) who reported the frequent contamination of dairy products by *Staphylococcus aureus*. The contamination of yoghurt by yeasts and molds has also been reported by several researchers (Pederson, 1979; Green *et al*., 1987; Foschino *et al*., 1993; Montagna *et al.,* 1998 and Moreira *et al.,* 2001). Green *et al.* (1987) and Foschino *et al.* (1993) have reported the presence of *Mucor spp* in commercial yoghurt sold in Lagos, Nigeria and Milan, Italy, respectively.

The presence of *Staphylococcus aureus* in the probiotic yoghurt may cause food poisoning. Staphylococcal food poisoning is a major type of food intoxication (Willey *et al.,* 2008). It is caused by the ingestion of improperly stored or cooked food in which *S.aureus* has grown.

S.aureus is very resistant to heat, drying, and radiation; it is found in the nasal passages and on the skin of humans and other mammals (Willey *et al*., 2008). It might have entered the probiotic yoghurt through these sources during the production process. The bacteria produce heat-stable enterotoxins that renders the yoghurt dangerous even though it appears normal. Thirteen different enterotoxins have been identified; enterotoxins A, B, C1, C2, D and E are the most common. These toxins appear to act as neurotoxins that stimulate vomiting through the Vagus nerve (Willey *et al*., 2008).

The presence of *Mucor spp* in sample 1 indicates contamination of the probiotic yoghurt. *Mucor spp* that have been isolated from yoghurt include *Mucor hiemalis* and *Mucor racemosus* (Foschino *et al*., 1993). The presence of these thermotolerant *Mucor spp* in yoghurt may lead to human infections such as Mucormycosis when ingested in amount that can cause the problem (Geo *et al*., 2007).

Bacillus spp was isolated from sample 2 (Batch B). Bacteria in the genus *Bacillus* forms endospores and are chemoorganotrophic and catalase positive (Willey *et al*., 2008). *Bacillus spp* belongs to the same class (Class Bacilli) as the two other probiotic constituent of the probiotic yoghurt (i.e. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) according to Bergey's manual (2nd edition).

The species of *Bacillus* that is known to cause food intoxication is *Bacillus cereus*. It can cause two distinct types of illness depending on the type of toxin produced: an emetic illness characterized by nausea and vomiting with an incubation time of 1 to 16 hours, and a diorrheal type, with an incubation of 4 to 16 hours.

CONCLUSION

The shelf life of the probiotic yoghurts studied is claimed to be 60 days by the manufacturers. However, according to Shah (2000), the normal shelf life of probiotic yoghurt should be 30 days. The presence of *Mucor spp* and *Staphylococcus aureus* in Sample 1(Batch A) after 49 days of storage indicates that the probiotic yoghurt expired before the expiring date on the label.

Sample 2 (Batch B) was analyzed after 11 days of storage. The isolation of *Bacillus spp* from Batch B may not necessarily indicate that the probiotic yoghurt is contaminated. This is because i.) *Bacillus spp* belongs to the same class as two of the four probiotic constituents in the yoghurt (i.e. *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) and ii.)The identification tests were not sufficient to reach definite conclusions but the result may also suggest contamination due to mishandling in the production process. It is suggested that care should be taken by the handlers of the product to avoid contamination in the future.

It is recommended that more studies be carried out in this area.

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