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MICROBIOLOGICAL QUALITY OF RE-PACKAGED SWEETS SOLD IN METROPOLITAN KANO, NIGERIA

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ABSTRACT

Some readily-available sweet samples comprising of three brands coded A, B and C re-packaged for sale in Kurmi market of metropolitan Kano, Nigeria were microbiologically examined for the load and type of bacteria and fungi. Samples from brand A had average aerobic bacterial and fungal counts of 5.40×10^2 cfu/g and 1.30×10^2 cfu/g respectively. Samples from brand B had average aerobic bacterial and fungal counts of 5.40×10^2 cfu/g and 1.00×10^2 cfu/g respectively while samples from brand C had aerobic bacterial and fungal counts of 1.30×10^2 cfu/g and 1.00×10^2 cfu/g and 6.80×10^2 cfu/g respectively. The moisture contents were 13.2%, 13.4% and 14.1% for the A, B and C brands of the sweets respectively. Cultural, morphological and biochemical characterization of the isolates showed the presence of two bacterial genera (Staphylococcus and Bacillus) and five fungal genera (Penicillium, Aspergillus, Mucor, Rhizopus and Fusarium) as the predominant contaminants. The average bacterial and fungal counts for the control were 6.00×10^1 cfu/g and 1.30×10^1 cfu/g respectively while the moisture content was 3.3%. The practice of re-packaging of industrial remnants should be discouraged as this predisposes the wholesome products to contamination.

Keywords: Microbiological quality, re-packaged sweets, Kurmi market, Kano.

INTRODUCTION

Sweets include both hard and soft confectioneries, which cover not only pure sugar concoctions but also include an array of tasty confectioneries containing sugar or similar substances with other compatible ingredients such as fruits, nuts or chocolates (Schultz, 1994), which have been solidified aseptically by industrial process. Such finished products called sweets are normally packaged aseptically by the manufacturers with minimum microbial contamination for public consumption. However, situations arise whereby retailers have to devise means of collecting the industrial remnants and re-packaging them for sale to unsuspecting public for consumption. This practice of re-packaging of industrial remnants presents a potential for contamination of the products at market places (Adesiyun, 1984). According to the Association of Food and Drug Officials (AFDO, 1990), simple packaging or re-packaging operations can bring about an opportunity for the contamination or recontamination with pathogens if strict aseptic conditions are not adhered to. According to Frazier and Westhoff (1978), sweets receive most of their contamination from their ingredients, although some contaminants may be added by unwrapped pieces by air, dust and handling. Additional contamination may come from equipment coming in contact with food from packaging materials and from personnel (Greenwood and Handhooper, 1983; Abdullahi et al., 2005; Mohammed et al., 2005; Rogo and Kawo, 2005; Aminu et al., 2006; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008). Generally, poor sanitary conditions and the environment being highly charged

with spoilage and pathogenic flora could be the source of contamination to food items exposed to it. Thus, retailers of food products, which include sweets, have been implicated in the spread of food-borne diseases (Adesiyun, 1984; Abdullahi *et al.*, 2005; Shamsuddeen and Ameh, 2008; Shamsuddeen *et al.*, 2008; Oyeyi and Lum-nwi, 2008; Wada-kura *et al.*, 2009). Various products have been implicated in food poisoning due to their quality, composition and general handling (Hans and Frank, 1979; Odeyemi, 1984). The present study aims at examining the microbiological quality of re-packaged sweets sold at Kurmi market in the metropolitan Kano, Nigeria with a view to assessing their microbiological fitness for human consumption.

MATERIALS AND METHODS Sample collection

This was carried out according to the method of Food and Agricultural Organisation (FAO, 1979; 1993). Three different brands of wholesome sweets namely milkose, tom-tom and splash coded as A, B and C respectively, re-packaged for sale to consumers were purchased from three sheds where sweet remnants from industries are re-packaged for sale to consumers in Kurmi market of Municipal local government area of Kano State, northern Nigeria.

Twenty-four (24) samples from each shed (already opened and tied for sale) were randomly and aseptically collected in a 150ml-capacity, widemouthed glass containers with air-tight fitting caps, which were previously disinfected with ethyl alcohol and autoclaved at 121°C for 15 minutes. The samples were immediately taken to laboratory for analysis.

Microbiological analysis of the samples *Inoculation of samples*

The inoculation of the samples was carried out using standard-plate count technique (FAO, 1993). Twentyfive grams (25g) of the sweet sample was aseptically weighed and introduced into 225 ml of previouslysterilized buffered peptone water (LAB M) while a wholesome beverage was used to serve as a control. This was achieved by aseptic opening of the cellophane bags (by wiping the tied mouth of the cellophane bags with ethyl alcohol) in which the sweets were tied. The mixture (solution) was homogenized by shaking vigorously to produce the sweet homogenate. The homogenate was serially diluted (10⁻¹ to 10⁻³) and 1.0 ml of each homogenate was inoculated into correspondingly labelled plates of nutrient (biotec) agar medium for bacteria and potato dextrose (LAB M) agar medium for fungi. The plates for bacterial counts were incubated at 37°C for 24 hours while those for fungal counts were incubated at room temperature (27±1°C) for 3-5 days. The numbers of colony forming units were counted, recorded and expressed in colony forming units per gram (cfu/g). In addition, selective and differential media (glucose blood agar, MacConkey agar, mannitol salt agar and peptone water) were also inoculated and incubated at 35°C for 18-24 hours.

RESULTS

The results of the microbiological analysis and moisture content of the re-packaged sweet samples purchased from Kurmi market in metropolitan Kano are presented in Table 1. The highest and lowest mean bacterial counts were 1.30×10^4 cfu/g and 5.40×10^2 cfu/g obtained in brands C as well as A and B respectively. The highest and lowest mean fungal counts were 6.80×10^2 cfu/g and 1.00×10^2 obtained

Cultural, morphological and biochemical characterization of the isolates

The method of Cheesbrough (2002) was used for the identification of the bacterial isolates. Colony appearance was observed and recorded while Gram's reaction and spore staining were carried out to ascertain the morphology and Gram's reaction-behaviour of the bacterial isolates. In addition, catalase and coagulase tests were carried out. For the identification of fungi, cotton-blue in lactophenol was used and the hyphae examined microscopically using X10 and X40 objective lens (Beneke and Stevenson, 1977).

Determination of moisture content

This was carried out in accordance with the method of Egan *et al* (1983) using gravimetric technique. Crucible dishes were oven-dried to a constant weight at 180°C for one hour after which they were taken into a desiccator for cooling to room temperature. The empty crucible was weighed (W_1). Five grams of the sweet sample was placed into the crucible and the whole set up was re-weighed and recorded as W_2 . The crucible containing the sample was then taken into a hot-air oven for 3 hours at 115°C after which it was transferred into a desiccator and allowed to cool at room temperature. The content was then weighed (W_3). The percentage moisture content was finally calculated using the following relationship:

% moisture =
$$\frac{W2 - W3}{W2 - W1} \times 100$$

in brands C and B respectively. The moisture content ranged between the highest and lowest of 14.1% and 13.2% obtained in brands C and A respectively. Cultural, morphological and biochemical characterization of the isolates showed the presence of two bacterial genera (*Staphylococcus* and *Bacillus*) and five fungal genera (*Penicillium, Aspergillus, Mucor, Rhizopus* and *Fusarium*) as the predominant contaminants (Tables 2 and 3).

Table 1: Mean bacterial and fungal counts and percent moisture contents of the re-packaged sweet samples

Brand code (n = 24)	Bacterial count (cfu/g)	Range of bacterial count (cfu/g)	Fungal count (cfu/g)	Range of fungal count (cfu/g)	Moisture (%)
Α	5.40 x 10 ²	6.0 x 10 ¹ - 2.0 x 10 ³	1.30 x 10 ²	2.6 x 10 ¹ – 2.9 x 10 ²	13.2
В	5.40 x 10 ²	2.0 x 10 ¹ – 1.6 x 10 ³	1.00 x 10 ²	1.0 x 10 ¹ – 2.4 x 10 ²	13.4
С	1.30 x 10 ⁴	$1.1 \times 10^2 - 6.0 \times 10^4$	6.80 x 10 ²	1.5 x 10 ¹ – 9.0 x 10 ²	14.1
Control	6.00×10^{1}	$0.0 \times 10^{0} - 2.0 \times 10^{1}$	1.30 x 10 ¹	$1.0 \times 10^1 - 2.0 \times 10^1$	3.3

Colony appearance	Gram's reaction	Spore	Catalase	Coagulase	Organism
On nutrient agar, colonies were grayish, granular discs, 2-3 mm in diameter. On blood agar, colonies produced very slight haemolysis. Smooth, circular, low convex, glistering and butyrous colonies, usually	Gram-positive bacilli	+	+	Non-detectable	<i>Bacillus</i> species
1-3 mm in diameter on MacConkey agar and blood agar media.	Gram-positive cocci	-	+	+	Staphylococcus aureus

Table 3: Cultural and morphological characteristics of the fungal isolates

Colony appearance	Morphology	Organism
Colonies were of a wide variety of colours (green, brown, black, grey,	Mycelium is septate, conidiophores were non- septate, each ending in a terminal, enlarged	
yellow, etc) depending on the colour of the conidia.	spherical swelling. Septate and multinucleate hyphae with sprinkler conidia.	Aspergillus species
Pink, purple/yellow, white and fuzzy	The macroconidia were spindle-shaped. Septate	
colonies. The colonies were fluffy and spreading.	mycelium bearing crescent conidia on the conidiophores	Fusarium species
Colonies were initially white or green (cotton wool-like), which later became darker (grey-black) as sporangia were produced.	Hyphae were thick and non-septate with large diameter. The sporangiophores were erect and sometimes unbranched and/or unbranched, which bear single sporangium containing a large number of spherical spores. Non-septate, thick hyphae with round columella and sporangia	<i>Mucor</i> species
Colonies varied in colour (depending on the species) from green, grey- green, blue-green or yellow-green. The colour of the colonies changed very often.	Mycelium with septate hyphae from which conidiophores arose, bearing brush-like conidia.	Penicillium species
Wholly white colonies similar to cotton wool, which grew rapidly on plate cultures with black pin heads, which later turned black and/or brown.	Mycelium had non-septate hyphae of large diameter. The cottony mycelium produced cluster of root-like structures called stalon and rhizoids.	<i>Rhizopus</i> species

DISCUSSION

From the results obtained in the present study, it was shown that the mean bacterial counts of the sweet samples from C brand obviously exceeded the maximum recommended standards by the International Commission Microbiological on Specification of Foods (ICMSF, 1978). According to this agency, the acceptable limit of mesophilic aerobic bacteria in dried food products should not exceed a maximum of 10^3 cfu/g. However, brands A and B had counts within the acceptable limits recommended by ICMSF (1978). On the other hand, all the results of the fungal counts from all the three brands analysed were within the acceptable limit. However, the counts are considerably high since no microorganism should be recovered in any food meant for human consumption (FAO, 1979; 1993; WHO, 2003). The generally observed high microbial counts in this study could be attributed to the influence of environmental factors on the microbial populations, which have been shown to play a significant role in affecting the quality of food products (Owhe-oreghe and Afe, 1993; Abdullahi et al., 2005; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Oyeyi and Lum-nwi, 2008; Wada-kura et al., 2009). The ways these products are handled in an open air environment are no exception. The re-packaging materials are also a possible source of contamination (Frazier and Westhoff, 1978) because they are ordinarily wrapped and the wrappers are not subjected to any bacteriostatic or fungistatic treatment. According to WHO (2003), a food is deemed to be adultered if its content is composed in whole or in part of any poisonous or deleterious substance, which renders its contents injuries to health.

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morphological The cultural, and biochemical characterization of the microbial isolates recovered from the re-packaged sweet samples analysed in this study indicated the presence of Staphylococcus aureus, Bacillus sp, Penicillium sp, Aspergillus sp, Mucor sp, Rhizopus sp and Fusarium sp as the predominant contaminants. Particularly important are the S. aureus and Bacillus sp. These are known causative agents of food poisoning and intoxication (FAO, 1979; Adams and Moss, 1995). The presence of these bacteria may be due to the unhygienic environmental conditions and poor handling. Various researchers (Adesivun, 1984; Abdullahi et al., 2005; Aboloma, 2008; Shamsuddeen and Ameh, 2008; Shamsuddeen et al., 2008; Oyeyi and Lum-nwi, 2008; Wada-kura et al., 2009) have reported that the presence of Staphylococcus aureus in food is an indication of environmental and human contamination. According to Wufem et al (2004), the presence of S. aureus might be attributed to contamination of foods through air, mouth and respiratory tract of the handlers. The recovery of S. aureus from the sweet samples examined in this study could be traced to the fact that it is abundant in human body especially as a normal flora of the skin. It is also reported to contribute 40-50% nasal carriers in humans (Ogbini and Omu, 1986; Uabol-Egbenni, 2003; Onukwubiri, 2005). Of particular importance is the ability of S. aureus to elaborate enterotoxins in foods, which are dangerous to human and other animal health (Grundy and Grundy, 1974; Hobbs, 1974; Frazier and Westhoff, 1978; Okafor, 1987; Wieneke et al., 1993). The presence of Bacillus species in this study might be due to poor handling. Several dried food samples have been reported to contain some of these organisms (Blackey and Priest, 1980; Aboloma, 2008; Frazier and Westhoff, 1978). Blackey and Priest (1980) reported that Bacillus species is common in soils and vegetation and has been isolated in several countries from wide variety of routine samples of food. The occurrence of this bacterium in the present study is therefore not surprising because of the way the products (sweets) are handled in an open market in a dusty and muddy environment. Its presence therefore could be due to the contamination from many sources, which may include soil, air and water. The organism might have come in during processing; an observation that goes to support Pederson (1979) according to whom spores of molds and Bacillus abound in air and water. The fungi isolated in this study are mostly contaminants. The surrounding air, packaging materials and the personnel concerned with the packaging processes could all serve as sources of these contaminants. This agrees with Aboloma (2008) as well as Akinyosoye and Nwosisi (1994) who isolated these organisms and reported that they could be contaminants from air or materials used in processing. The isolation of these organisms gives serious cause for concern because Aspergillus species is specifically known to produce mycotoxins (Hobbs, 1974; Frazier and Westhoff, 1978; Alexopoulos and Mims, 1979; Hans and Frank, 1979; Adams and Moss, 1995), which cause food intoxication

in man and other animals. The high fungal counts suggest the presence of fermentative organisms (Okafor, 1987; Pederson, 1979; Zocklein, 1990) even though no yeast was isolated. This is because *Aspergillus* species is capable of utilizing an enormous variety of substances as food because of the large numbers of enzymes it produces. It is capable of causing spoilage of food products containing high sugar concentration (Wilkie, 1998). Thus, the presence of *Aspergillus* species in sweet samples examined in the present study could result in the production of toxic substances (mycotoxins), which could lead to health hazards for the consumer (Frazier and Westhoff, 1978; Weinzirl, 1992).

The moisture contents of the products examined in this study are indicative of the fact that the bacteria and fungi might have absorbed some moisture from the surrounding environment during repackaging processes. This is because the successful growth of these micro-organisms depends upon their getting an adequate supply of moisture (Mansrelt, 1964; FAO, 1979; Adams and Moss, 1995). This could allow pathogens to develop by multiplying to levels where they could cause food poisoning (Abdullahi *et al.*, 2005). With the highest recorded moisture content of 14.1% (C brand) compared with the control (3.3%), there is a good possibility of bacterial multiplication.

CONCLUSIONS AND RECOMMENDATIONS

The average counts for bacteria of the sweet brands examined are generally below the maximum allowable limit in dried foods to be marketed for consumption (10^3 cfu/q) except for the C brand while the fungal counts are all within acceptable limit. However, the average ranges obtained for the bacteria indicated a public health concern as they showed counts far above this limit. These high counts are suggestive of heavy bacterial contamination of the sweets during handling since the sweets might have absorbed adequate supply of moisture, which could have contributed to the development as well as multiplication of these contaminants. From the results of the study, it is evident that the practice of collecting industrial remnants and re-packaging them to sell to consumers could expose the consumers to health problems. This should completely be discouraged so as to produce consistently-consumed sweets with high quality and low microbial load. Only sweets that are aseptically processed should be sold. These could be achieved by:

- (a) protecting all personnel, the surrounding environment and the packaging materials from contamination with dust and/or other sources of microorganisms during handling; and
- (b) proper washing and sanitization of all equipment and other utensils so as to prevent the spread of diseases (infections) as has been campaigned by the National Agency for Food and Drug Administration and Control (NAFDAC).

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