

# MICROBIAL HAZARDS ASSOCIATED WITH COMMERCIAL CANDIES SOLD IN NIGERIAN MARKETS

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## ABSTRACT

The increase in demand and supply of candies in Nigeria necessitate thorough microbiological investigation to verify the possible food hazards associated with their consumption in Nigeria. This research aim at determining the microbiological food safety hazards associated with commercial candies sold in Nigeria using Akure as a case study. Questionnaire was developed and distributed to respondent to determine the commercial practices of candy sellers in Akure metropolis, average manufacturing date, expiry date, batch number, packaging, storage condition, display condition and date of assessment were determined and this formed the criteria on which the candies were acquired. Microbiological examination of the candies included test for coliforms bacteria, microscopic filamentous fungi and yeasts, *Salmonella sp.* and *Staphylococci*. Results obtained (total viable counts, coliform, mould, *E. coli*, *Staphylococcus aureus* and *Salmonella*) based on points of display (POD), difference in packaging (DIP), duration of storage (DOS), and samples with 95-100% sugar content (SSC). For POD ranges from  $1.0 \times 10^1$ - $2.4 \times 10^2$ ;  $8.4 \times 10^2$ ; nil; nil;  $2.4 \times 10^2$ ;  $1.0 \times 10^1$ - $9.6 \times 10^3$ , while DIP -  $1.0 \times 10^2$ - $2.0 \times 10^2$ ;  $2.0 \times 10^1$ ;  $3.2 \times 10^2$ - $4.4 \times 10^2$ ; nil; nil; nil; DOS -  $9.7 \times 10^3$  cfu/g; nil; nil; nil; nil and SSC-  $4.7 \times 10^2$ ;  $1.0 \times 10^1$ ;  $1.0 \times 10^1$ ;  $1.0 \times 10^1$ ;  $1.2 \times 10^3$ - $6.8 \times 10^3$  cfu/g respectively. Milk candy, among the samples contained the highest numbers of organisms and this may probably have resulted from inadequately pasteurized raw milk used and processing conditions.

**Keywords:** Candies, Bacteria, Mould, hazards, Processing conditions, Questionnaire

## INTRODUCTION

Candy is a confection made from a concentrated solution of sugar in water, to which flavorings and colorants have been added. Candies are usually in numerous colours, varieties and have a long history. They could be soft, chewy, hard and brittle, there are a wide range of chocolate candy (Stroud, 2008). Candies that contain peppermint and mint have digestive benefits and create defense against irritation of the bowel (Viegas, 2008). Scholey. (2012) reported that chewing gum can also increase insulin level in anticipation for food. When eaten in moderation, dark chocolate are beneficial to the health such as reducing the risk of heart disease and increases longevity. Chocolates have been reported to contain vitamins and minerals such as calcium, magnesium, and sodium as well as antioxidants (Stroud, 2008). Candies serve as snacks to children during school periods and parties.

Due to the high content of most candies, they often serve as good medium for growth of microorganisms, hence

several authors have reported the presence of both pathogenic and spoilage microorganisms in candies. Most of these microorganisms contaminate candies through their ingredients, although some may be contaminated by packaging materials, personnel, exposure to air, dust and equipment. (Mohammed et al., 2005; Shamsuddeen et al., 2008). Chocolate creams are candies that have been reported to undergo microbial spoilage. *Clostridium spp.* especially *Clostridium sporogenes*, *Leuconostoc mesenteroides* and *Bacillus* which get into the products through starch, sugars and into other ingredients have been reported to be causative organisms (Owhe-oreghe and Afe 2003). Adequate supply of moisture and availability of other essential nutrients (other than carbohydrates), are required for the successive growth of these organisms (D'Aoust, 1977). Deviations from good manufacturing practice, in-line and finished product contamination during manufacture and the use of contaminated raw materials are also possible routes of contamination of chocolate by *Salmonella* bacteria (D'Aoust, 1977, Baylis et al., 2001). Rogo and Kawo (2005) reported that microbial hazards of concern are bacteria, viruses and parasites and these have formed the top food threat especially to children

and people with compromised immune system who are the greatest risk of falling ill to the sicknesses caused by the microorganism, hence the need to evaluate the microbial hazards associated with the consumption of these candies. This study has determined the market status and condition of storage of commercial candies, evaluated the microbiological quantity of commercial candies sold, and determined the microbiological risk in the possible consumption of the candies in Akure metropolis.

## **MATERIALS AND METHODS**

### **Materials**

A market survey was carried out using the Akure main market, Ondo state, Nigeria as a case study and this was to verify the types of candies that are sold in the market. A questionnaire was developed and distributed to respondents to determine the commercial practices of candy sellers in Akure metropolis. The questionnaire was designed to determine average manufacturing date, expiry date, batch number, packaging, storage condition, display condition and level of patronage of different brands of candies. Information gathered formed the basis on which candies were acquired for microbiological information.

### **Acquisition of Samples**

Based on the survey, candy samples were purchased using categorizations such as; Point of display, packaging, duration in storage, sugar content. It was ensured that the candies were taken from both freshly opened cartons that are stored properly and also those that have been stored for a long time and not properly packaged. The samples were brought to the laboratory and stored in a good condition to avoid further possible contamination and analyzed at the shortest time.

### **Microbiological Analysis**

#### **Sterilization of media and glass wares**

All glasswares were thoroughly washed with detergents, rinsed with clean water and allowed to drain off before being sterilized in an autoclave at 121°C for 15 min at 15psi. The bench top was also swabbed with 70% alcohol, Nutrient (2.8g/100ml), potato dextrose (3.9g/100ml), eosin methylene blue (3.72g/100ml), mannitol salt (10.8g/ml), MacConkey (5.2 g/100ml) and deoxycholate (4.52g/100ml) agars were prepared according to manufacturers' specifications. All conical flasks containing agars were covered with cotton wool and wrapped with foil paper to prevent evaporation during sterilization. The flasks were autoclaved at 121°C

for 15 min at 15psi. After autoclaving, the agars were allowed to cool before use.

### **Serial dilution/pour plate technique**

Serial dilution techniques were carried out as described by Olutiola *et al.* (1991). Molten agars were poured aseptically into the petri dishes that have been labelled as followed by Olutiola *et al.* (1991). The PDA plates were acidified using lactic acid before pouring the agars to inhibit bacterial growth. All bacterial plates were incubated at 37°C for 24 h for in an inverted position while fungal plates were incubated at ambient temperature (25°C ±2) for 72 h.

### **Purification and preservation of isolates**

Colonies were randomly picked and sub cultured onto fresh agar surfaces by streaking using an inoculating loop. This was determined according to Feng (1996).

### **Identification and Characterization of isolates**

#### **Colonial characteristics**

The colonies were observed by direct observation, characteristics such as shape, size, opacity, edge, elevation and colour of the colonies on agar surface were observed as described by Olutiola *et al.* (1991).

#### **Morphological characteristics of the isolates**

Morphological characteristics of the cells were observed in a stained preparation using the gram staining procedure as described by Olutiola *et al.* (1991).

#### **Biochemical characteristics**

##### *Indole Production*

Each test organism was aseptically inoculated into its appropriately labelled Tryptone water as described by Feng (1996)

##### *Growth in Salt (NaCl)*

Growth in different salt concentrations was tested by preparing salt agar (nutrient agar and salt) and adding the required amount of NaCl (7%, 10%,) as described by Olutiola *et al.* (1991).

##### *Urease Activity*

Basal Urea medium containing peptone (0.1g), NaCl (0.5g), KH<sub>2</sub>PO<sub>4</sub> (0.2g), glucose (0.1g), phenol red (0.6m), sugar (2g) and distilled water (100ml) was prepared and distributed into bottles, sterilized at 121°C for 15 min and cooled to 45-50°C. The Urea solution was

sterilized by membrane filtration, and added to the basal medium to give a final concentration of 2% urea. The bottles were slanted and the contents allowed to solidify. A little of the isolate was streaked over the surface of the agar and a control of the basal medium containing no added urea was inoculated. The bottles were incubated at the optimum temperature and examined daily for up to 7 days for the production of alkali this is indicated by a red colouration (Olutiola *et al.*, 1991).

#### Sugar Fermentation

One percent (1%) peptone water, 0.1% of NaCl and 1.0% of each of the sugars were weighed into a 250ml of conical flask then 100ml of water was added, to this medium an indicator was added (phenol red) the medium was distributed into bottles and Durham tubes were inverted in each tube, sterilized at 121°C for 15 min and cooled to 45-50°C. One tube of Each carbon type was inoculated with the same organism, this was incubated at optimum temperature, an un-inoculated control was incubated and was examined daily (Olutiola *et al.*, 1991).

#### Hydrolysis of Casein

Litmus milk was prepared by weighing 10.3g of litmus milk, 2.8g of nutrient agar, 100ml of water were weighed into a conical flask and homogeneity was ensured, it was autoclaved at 121°C for 15min and was carried out according to Olutiola *et al.*(1991).

## RESULTS AND DISCUSSION

### General Information on the Commercial Practices of Candy Sellers in Akure South Ondo State, Nigeria.

Table 1 shows the information gathered on the commercial practices of candy sellers in Akure South, Ondo State Nigeria. It was observed that 35% of the respondents usually displayed their candies under direct sunlight for sale, while 40% displayed theirs in shops with fans. Only a very small number of the respondents (about 25%) displayed their candy in air-conditioned shops. The display of candies under direct sunlight, even though it is a common display condition for candies sold in Akure metropolis, may not be very good because it may absorb moisture and lead to formation of mold (Stroud, 2008).

### Microbial Loads of Candies

Table 2 shows the microbial loads of the candies. Based on differences in packages, counts ranged from  $1.0 \times 10^1$  to  $2.0 \times 10^2$  cfu/g total viable count,  $3.2 \times 10^2$  cfu/g to  $4.4 \times 10^2$  cfu/g mould,  $2.0 \times 10^1$  cfu/g coliform for candies packed in nylon, bowls, plastic toys and sulphite paper

respectively, no *E. Coli*, *Staphylococcus aureus* and *Salmonella* was found in the samples which may be due to contamination during processing and handling conditions, the moulds present in the samples may be as a result of condensation of moisture in water-impermeable packages as this is seen in products packed in bowls, sulphite paper and plastic toys. Based on the duration of storage, total viable count was  $1.2 \times 10^3$ . Count was  $9.7 \times 10^3$  *Staphylococcus aureus*. The samples did not contain any coliform, *E.coli*, *Salmonella* and mould for candies freshly supplied while candies close to expiration had no counts at all. This is an indication that the microbial count may not have been significantly affected by post storage conditions; therefore the microbial load may have been contributed during processing as a result of unhygienic processing condition and the raw materials used. This can be seen in the case of milk candy that had the highest microbial load (Table 2). Raw milk and other milk-based products naturally contain both beneficial and pathogenic bacteria. Sheehan, (2005) reported that *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Campylobacter jejuni* and *E.coli* O157:H7 are pathogenic bacteria that may be found in milk. Total viable counts of the candies with respect to display condition, ranged from  $1.0 \times 10^1$  to  $2.4 \times 10^2$  cfu/g,  $8.4 \times 10^2$  cfu/g coliform,  $2.4 \times 10^2$  cfu/g *Staphylococcus aureus*,  $9.6 \times 10^3$  to  $1.0 \times 10^1$  cfu/g *Salmonella*,  $1.0 \times 10^1$  cfu/g mould for candies stored in fanned shops, under direct sunlight and air-conditioned shops respectively. The higher microbiological count of samples in the shops with fan as compared to others is an indication that that storage condition favored microbial growth. Usually the shops are open, so microorganisms are blown in from the surrounding air and spread on the samples. Secondly, these shops are usually not too cold nor hot (usually around temperature of  $27^\circ\text{C} \pm 2$ ) and mesophiles' growth is usually supported at this temperature. The relatively high numbers of *Salmonella* and coliforms may be more related to processing/ packaging conditions and may not be as a result of storage in fanned shops. The absence of moulds in the samples is an indication that the storage environment was not humid to support the growth of moulds. Based on sugar content the counts ranged from  $1.2 \times 10^3$  to  $6.8 \times 10^3$  cfu/g *salmonella*,  $4.7 \times 10^2$  total viable count,  $1.0 \times 10^1$  cfu/g coliform,  $1.0 \times 10^1$  cfu/g *Staphylococcus aureus*,  $1.0 \times 10^1$  cfu/g mould, the sample did not contain *E. coli*. Kacaniova *et al.* (2011) reported that the major hazards may come from ingredients because candies have low water activity and is generally free from microbial contamination. Hence the ingredients may have contributed to the high numbers of *Salmonella/Shigella*.

**Colonial, Morphological and Biochemical Characteristics of Organisms Isolated from the Candies**

Table 3 shows the colonial, morphological and biochemical characteristics of bacterial isolated from the candies while moulds isolated and identified are *Geotrichumaibidiun* and *Fusarium oxysporum*, the bacteria isolated were majorly Gram positive, non sporing, rod and cocci. 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. The presence of *Staphylococcus aureus* in food has been reported by many researches as an indication of environmental and human contamination. *Staphylococcus aureus* found in the samples may be due to the fact that could be to the fact that it is a normal flora of the skin and can spread enterotoxins in foods, which are dangerous and cause hazards to human health (Wieneke et al., 1993).

The presence of *Bacillus species* in this study might be due to poor handling as they have been reported to be

found in several dried food samples (Aboloma, 2008).

**Predominance and Occurrence of Microorganisms in the Candy Samples**

Table 4 shows Predominance and occurrence of microorganisms in the Candy Samples. *Enterobacter aerogenes* occurred in toffees and milk candy, *Bacillus lentus* occurred in lollipops and parago. *Staphylococcus epidermis*, *Acinetobacter parapertusis*, *Staphylococcus aureus*, *Corynebacterium murium* and *Bacillus licheniformis* occurred in lemon drops. *Clostridium carius*, *Micrococcus Roseus*, *Bacillus lentus*, *Citrobacter frerundii*, *Shigella sonnei* and *Salmonella spp* occurred in milk candy. *Geotrichumaibidiun* occurred in caramel and marshmallow while *Fusarium oxysporum* occurred in caramel, lollipops, tomtom, plastic children toy candy, parago and lemon drops. This agrees with Aboloma (2008) and Akinyosoye (1994) who isolated these organisms and reported that they could be contaminants from air or materials used in processing which could lead to health hazards for the consumer.

**Table 1: General information on the commercial practises of candies sellers in Akure South Ondo State, Nigeria.**

Parameters	Packaging/ wrapper	Points of display	Duration in storage	Sugar content	Preference
Mostly used	Nylon (40%), candies Plastic toys (20%)	Displayed in Fan shops (40%)	1-12 months (40%)	100% (70%)	Toffees, lollipops, lemon drops, peppermints (60%)
Fairly used	Bowls (15%), sulphite paper (15%)	Displayed under the sun (35%)	1-24 months (35%)	95% (20%)	Marshmallows, caramels (25%)
Least used	Cartoon (10%)	Displayed in air conditioned store (25%)	1-36 months (25%)	75% (10%)	Fudge, nougats (15%)

Table 4.2: MICROBIAL LOADS (cfu/g) OF CANDIES ACCORDING TO GROUPS

Groups (based on commercial practices)	TVC	Coliform	<i>E. col</i>	<i>Staphylococcus aureus</i>	<i>Salmonella/s higella</i>	Mould
<b>Packaging/wrapper</b>						
Nylon	2.0 × 10 <sup>2</sup>	Nil	Nil	Nil	Nil	Nil
Bowl	Nil	Nil	Nil	Nil	Nil	3.3 × 10 <sup>2</sup>
Plastic toys	Nil	Nil	Nil	Nil	Nil	4.4 × 10 <sup>2</sup>
Sulphite paper	1.0 × 10 <sup>1</sup>	2.0 × 10 <sup>1</sup>	Nil	Nil	Nil	3.2 × 10 <sup>2</sup>
<b>Duration of storage</b>						
Freshly supplied	1.2 × 10 <sup>3</sup>	Nil	Nil	9.7 × 10 <sup>3</sup>	Nil	Nil
Close to Expiration	Nil	Nil	Nil	Nil	Nil	Nil
<b>Point of display</b>						
Displayed in Fan shops	2.4 × 10 <sup>2</sup>	8.4 × 10 <sup>2</sup>	Nil	2.4 × 10 <sup>2</sup>	9.6 × 10 <sup>3</sup>	Nil
Displayed in airconditioned shops	1.0 × 10 <sup>2</sup>	Nil	Nil	Nil	1.0 × 10 <sup>1</sup>	1.0 × 10 <sup>1</sup>
Display under Sun	1 × 10 <sup>1</sup>	Nil	Nil	Nil	9.0 × 10 <sup>2</sup>	Nil
<b>Sugar content</b>						
100%	Nil	1.0 × 10 <sup>1</sup>	Nil	Nil	1.2 × 10 <sup>3</sup>	Nil
95-100%	4.7 × 10 <sup>2</sup>	1.0 × 10 <sup>1</sup>	Nil	1.0 × 10 <sup>1</sup>	6.8 × 10 <sup>3</sup>	1.0 × 10 <sup>1</sup>
75-95%	Nil	Nil	Nil	Nil	Nil	Nil

**Table 3 Colonial and morphological characteristics of organisms isolated from the candies**

Isolate code	Colour	Gram reaction	Shape of cells	Spore location	Urease	Casain	Indole	Glucose	Mannitol	Sucrose	NaCl (7%)	NaCl (10%)	Probable Microorganism
1	Cream	+	Short rods	No spore	+	-	+	+	+	+	-	-	<i>Corynebacterium</i>
2	Cream	-	Long rods	Central	-	-	+	+	+	-	-	-	<i>Enterobacter aerogenes</i>
3	White	+	Cocci	No spore	+	+	+	+	+	+	+	-	<i>Staphylococcus aureus</i>
4	Cream	+	Long rods	No spore	+	-	+	+	+	+	-	-	<i>Bacillus lentus</i>
5	Cream	+	Cocci	No spore	+	+	+	+	-	+	+	-	<i>Micrococcus roseus</i>
6	White	+	Short rods	Central	+	+	-	+	+	+	-	-	<i>Clostridium carius</i>
7	Cream	+	Cocci	No spore	+	-	-	+	+	+	-	-	<i>Staphylococcus epidermis</i>
8	Cream	-	Long rods	No spore	+	+	+	+	+	+	+	-	<i>Acinetobacter parapturtusis</i>
9	Cream	+	Short rods	Central	+	+	+	+	+	+	-	-	<i>Bacillus licheniformis</i>
10	Cream	-	Short rods	No spore	+	-	-	+	+	+	-	-	<i>Citrobacterferundii</i>
11	Cream	+	Short rods	Central	+	+	-	+	-	+	-	-	<i>Bacillus cereus</i>
12	Cream	-	Short rods	No spore	-	-	-	-	+	+	-	-	<i>Shigellasomei</i>
13	Cream	-	Long rods	Terminal	-	-	-	+	+	-	-	-	<i>Salmonella spp</i>

Table 4 Predominance and Occurrence of Microorganisms in the Candy Samples

Microorganisms	100% Sugar							95% Sugar		
	Toffees	Caramel	Lemon drops	Lollipop	Peppermint	Milk candy	Parago	Candies in Plastic toy	Marshmallows	
<i>Corynebacterium</i>	x	x	✘	x	x	x	x	x	x	
<i>Enterobacter aerogenes</i>	✘	x	x	x	x	✘	x	x	x	
<i>Staphylococcus aureus</i>	x	x	✘	x	x	x	x	x	x	
<i>Bacillus lentus</i>	x	x	x	✘	x	x	✘	x	x	
<i>Micrococcus roseus</i>	x	x	x	x	x	✘	x	x	x	
<i>Clostridium carius</i>	x	x	x	x	x	✘	x	x	x	
<i>Staphylococcus epidermis</i>	x	x	✘	x	x	x	x	x	x	
<i>Acinetobacter paraptusis</i>	x	x	✘	x	x	x	x	x	x	
<i>Bacillus licheniformis</i>	x	x	✘	x	x	x	x	x	x	
<i>Citrobacter ferundii</i>	x	x	x	x	x	✘	x	x	x	
<i>Salmonella spp</i>	x	x	x	x	x	✘	x	x	x	
<i>Bacillus cereus</i>	x	x	✘	x	x	x	x	x	x	
<i>Shigella sonnei</i>	x	x	x	x	x	✘	x	x	x	
<i>Geotrichum albicandidum</i>	x	✘	x	x	x	x	x	x	✘	
<i>Fusarium oxysporum</i>	x	✘	✘	✘	x	x	✘	✘	✘	

## CONCLUSION

The average counts for bacteria of the investigated candy brands examined are generally below the maximum allowable limit in dried foods to be marketed for consumption ( $10^3$ cfu/g). However, the average ranges obtained for the coliforms and *E.coli* indicated a public health concern as they showed counts that are high. These high counts are suggestive of heavy bacterial contamination of the candies during handling. The issue of safety and wholesomeness (safety) of food plays a special role in the prioritization of control during manufacture and handling of food. Questions of quality and wholesomeness of food, together with regard to environmental protection are becoming increasingly of concern not only among experts from various professions and disciplines, but also the general public

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