

# MICROBIAL CONTAMINANTS IN COCOA POWDER SAMPLES IN SOUTH – WEST NIGERIA

<sup>1</sup>Jayeola, C.O and <sup>2</sup>Oluwadun, A.

<sup>1</sup>*Cocoa Research Institute of Nigeria (CRIN), Ibadan, Oyo State, Nigeria.*

<sup>2</sup>*Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.*

## ABSTRACT

Cocoa powder (CP), which is the major ingredient of cocoa-based beverages, is obtained from cocoa cake in a process involving hydraulic pressing of cocoa butter from fermented and roasted cocoa beans. Cocoa powder is presently being consumed as a health drink because of the presence of flavonoids in it. Evidences have shown that cocoa flavonoids exert powerful antioxidant properties by boosting immune responses and also the presence of procyanidins in cocoa protects the body against free-radical. This study was carried out to investigate the microflora of twenty four brands of cocoa powder samples bought from different sources in South-West Nigeria with a view to determining their food safety.

A total of 360 samples of 24 brands of CP were analyzed for this experiment. Moisture content and pH were determined using recommended standard methods by Association of Official Analytical Chemist (AOAC). The viable bacteria counts were determined using cultural, microscopic and biochemical methods. Student t-test and ANOVA were employed in the statistical analysis of the data.

The result showed variation in the percentage moisture content as well as pH values from one CP to another. The pH values ranged from 6.4 to 7.4 while the moisture content of the CP fall between 0.80 and 1.86%. Percentage occurrence of predominant aerobic bacteria species were *Bacillus cereus* 33.3%, *Micrococcus acidophilus* 25%, *Staphylococcus aureus* and *Bacillus subtilis* each with 16.7% with *Bacillus macerans* 8.3% as the least encountered bacterium, the presence of coliform organisms were not found on all the samples analyzed.

The study showed that CP, which forms the bulk ingredient of cocoa-based beverages, can be a possible source of microbial contaminant to the beverage when carelessly handled.

**Key words: Cocoa powder, Microflora, Intrinsic factors, Food Safety, Bacteria.**

## INTRODUCTION

Food safety is usually determined by the absence or presence of pathogenic organisms, or their toxins, and the number of pathogens, with their expected or destructive agents (Ogunledun, 2007). The level of spoilage microbes reflects the microbial quality, wholesomeness, of a food product as well as the effectiveness of measures used to control or destroy such microbes (Pierson and Smoot, 2001).

Food borne disease and microbial spoilage of foods result from the failure of or inability to control micro organisms, at one or more stages of food chain from raw material production to consumption of the final product. Specifically, the microbiological tools are used to assess the safety of food, adherence to Good Manufacturing Practices (GMPs), the keeping quality (shelf life) of certain perishable foods and the utility (suitability) of a food or ingredient for a particular purpose (NRC, 1985). This experiment is therefore aimed at isolating the microflora associated with cocoa powder and do microbial count using standard plate count method and also to identify to species level bacteria flora of the CP using biochemical methods.

## MATERIAL AND METHOD

### Samples collection

Twenty four (24) brands of different cocoa powder circulating within Nigeria were analyzed. Each sample was collected by making three visits to the cocoa processing companies at three months interval.

### Pre-pour Plate method was used to plate out the samples:

1ml of each dilution was discharged into the centre of the appropriate Petri-dish. 10ml of molten each of the different media used was poured and rapidly but carefully mixed by a combination of to-and-fro and circular (clockwise and anti-clockwise) movements for about ten seconds. The plates were allowed to cool and set. They were inverted and incubated at 37°C for 24-48hours. Only plates which contain between 30 - 300 colonies were counted with Quebec colony counter and the number obtained was multiplied by the dilution factor to obtain the viable counts per ml of the original sample. **Characterization of Bacteria being isolated** Bacteria isolates were identified by morphological and biochemical tests as described in Bergey's Manual of determinative bacteriology. All the isolates were cultured on the prepared medium in duplicates and incubated aerobically at 37°C. The colonies were observed on the agar medium plates while the cell morphology was observed microscopically after staining. Various biochemical tests were carried out on the bacterial isolates for possible identification.

## RESULTS AND DISCUSSIONS

The cocoa powder brands consists of gram positive rods, gram positive cocci, mold and yeast with isolation percentages of 35.5%, 16.5%, 37.5% and 12.5% respectively. The highest occurrence of the microbes is recorded for mold. However, most of the cocoa brands are within the NAFDAC specification of  $1 \times 10^3$  (NAFDAC, 2005). Most brands with higher microbial load are recorded for brands that were purchased locally in open market within the country. The least microbial load was  $1 \times 10^2$  cfu/g and highest was  $2 \times 10^4$ . *Bacillus cereus* has the highest percentage isolation rates of 33.3%, *Baccillus subtilis* 16.7%, *Baccillus macerans* 8.3%, *Staphlococcus aureus* 16.7% and *Micrococcus acidophilus* 25%. The different cocoa powder with their correspondence Moisture content and pH respectively, the values for pH are within the range of 6.4 and 7.0. The percentage

mean moisture varies between 0.6 to 1.9%. This moisture contents were below the NAFDAC recommended maximum allowable moisture content of 5% in cocoa powder. The relationship between moisture contents (%) of cocoa powder and total bacterial counts (log cfu/g) showed a significant positive correlation ( $r = + 0.362$ ,  $P < 0.05$ ) was observed between the two parameters, with a linear equation of  $y = 0.52 + 2.93x$ . Regression determinant showed that moisture content is responsible for 13per cent increase in total viable bacterial counts.

The relationship between pH of cocoa powder and total bacterial counts (log cfu/g) showed an insignificant positive correlation ( $r = + 0.176$ ,  $P > 0.05$ ) was observed between the two parameters, this indicated that pH is not a determinant factor for bacteria growth in cocoa powder.

### **CONCLUSION**

*Bacillus species*, *Micrococcus acidophilus* and *Staphylococcus aureus* were the only bacteria isolated from the cocoa powder brands. This study revealed that the level of contamination observed in this study was mainly from small companies who buy the cocoa cake from the multinational companies and grind them to powder before selling them and also from open market purchases of cocoa packaged for caterers that use them in cake preparation. The extent of contamination of cocoa powder depends majorly upon the initial microbiological quality of the product and the level of aseptic precaution used during handling. The low moisture content of the samples had really contributed to the stability of the cocoa powder samples.

### **REFERENCES**

Ogunledun, 2007): Incidence of microbial contaminant and nutrient composition of selected cocoa based beverages in Ibadan, Nigeria, PhD Thesis, University of Ibadan.

(NRC, 1985): National Research Council. An evaluation of the microbiology criteria for food ingredient Washington DC National Academy Press. 1-69