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EFFECTS OF CHLORINE AND TEMPERATURE ON YEASTS ISOLATED FROM A SOFT DRINK INDUSTRY.

O. NWAIWU, M. I. NWACHUKWU and N. C. AMADI

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ABSTRACT

Yeasts isolated from sugar and filling valves in a bottling process were exposed to different chlorine concentrations and various high temperatures. It was found that growth of yeasts decreased with increase in chlorine concentration. The maximum chlorine concentration that inhibited both types of yeasts was 60mg/l while the minimum inhibitory concentration was found to be 40mg/l. Also with temperature increase yeast growth decreased. Both types of yeasts were best inhibited at a temperature of 80°C. Although filling valve yeasts were more susceptible to chlorine and temperature than sugar yeasts, correlations (p=0.01) observed for sugar and filling valve yeasts in the reaction with chlorine and heat indicate similarity and suggest that they may have the same cellular characteristics.

KEYWORDS: Yeasts, Chlorine, high temperatures, sugar, filling valves.

INTRODUCTION

Over the years, yeasts have taken over from bacteria as a tool for application in biotechnology and molecular biology research. It has been used extensively in industries, agriculture, and medicine e.g. brewer's yeast, baker's yeast and biofungicidal yeast. Other genetically modified yeasts are used in treatment of diabetes, high cholesterol level, and injury (Betz *et al.*, 1981) and O'Connor (1997). Researchers have been able to manipulate and direct yeast metabolism producing the desired products. Recently yeasts that have the same genetic sequence with humans have been developed.

However, during production of soft drinks, yeast is undesirable (Nwaiwu and Ibekwe, 2002, Tiina and Sandholm, 1989). It brings about spoilage by causing off-taste and bad appearance. When this happens it results in product recall from trade which can cause a company to lose product worth millions of naira and also put the image of the company at stake.

High yeast count from bottling equipment indicates that the equipment is not well cleaned and sanitized while low yeast count in a soft drink production environment demonstrates that manufacturers control the production environment and processes. It also shows that wholesome products are being delivered (Nwaiwu and Ibekwe, 2003).

The aim of this work therefore, is to determine the ideal temperature and chlorine concentration that would inhibit the growth of yeasts isolated from granulated sugar and filling valves of the filling machine during the process of bottling carbonated soft drink.

MATERIALS AND METHODS

Materials and methods used for this work were based on the methods used in the Nigerian bottling company, Owerri. The materials include basic glass wares in the laboratory for microbiological analysis. Glass wares were sterilized at 121oC for 15 minutes. Media used include Schaufus pottinger media (Satorius) which is selective for yeasts and mold. It is an artificial media prepared by adding 5mls of sterile water on nutrient pads of the media.

Physiological saline used for preparation of yeast stock cultures was prepared by dissolving 85 grams of sodium chloride in 1 liter of sterilized distilled water. Also Malt extract agar (LABM ™) was prepared according to manufacture's instructions and used to reconstitute the yeast cultures to confirm the presence of the organism in the stock solution.

Isolation of Yeasts and Identification of Yeasts

The yeasts used for this study were isolated from filling valves of the gravity filling machine and granulated sugar used for syrup preparation using the methods described in Anonymous (2002). Swab sticks were used to swab the filling valves after a production run. It was then introduced into a test tube containing 20mls of distilled water. It was left standing for 10 minutes after which the swab stick was discarded and the water poured into a membrane filtration apparatus. Vacuum was applied to draw the water through a 0.65 micron cellulose nitrate filter paper. After filtration, the filter paper was placed on Schaufus pottinger media (Satorius) and incubated at 30° C for five days.

For granulated sugar 10g was dissolved in 100mls of sterile distilled water after which it was filtered using the membrane filtration procedure described above.

Morphological characteristics of colonies that emerged from the samples after three days incubation were gram-stained and then compared to yeast colonies described in NBC (2002) and Cheesebrough (1985).

Preparation of Yeast Stock Culture and confirmation of yeast presence in stock solution

A loop full of the yeast isolated from sugar was introduced into a universal bottle containing 30mls of physiological saline and then stored at 8°C. The same procedure was repeated for filling valve yeasts.

Before using the stock culture for further tests, the presence of yeasts was confirmed by inoculating a loop full of the stock solution on malt extract agar (LAB M ™) by streaking. Colonies that emerged after 3 days were identified and compared to the colonies isolated earlier.

Effect of Chlorine on Yeast

Different concentrations of chlorine as described in Anonymous (2002) were prepared. Concentrations prepared included 10,20,30,40, 50 and 60mg/l.

McCartney bottles were then filled with 19mls of each chlorine preparation in duplicate after which 1ml of the sugar yeast stock solution was added. The 20ml mixture was allowed to stand for 30 minutes and filtered using membrane filtration and then plated on Schaufus pottinger medium (Satorius). The plates were then incubated at 30°C for 5 days after which colonies that emerged were counted with a colony counter (Anderman) and expressed in 20cfu/ml. The same

✉ **O. NWAIWU,** Department of Industrial Microbiology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria
M. I. NWACHUKWU, Department of Microbiology/ Industrial Microbiology, Imo State University, Owerri, Imo State, Nigeria
N. C. AMADI, Department of Microbiology/ Industrial Microbiology, Imo State University, Owerri, Imo State, Nigeria

Table1. Correlation analysis of sugar and filling valve yeasts.

Chlorine concentration(ppm)	Correlation Coefficient (%)
10	98
20	99
30	94
40	94
Temperature °C	
50	99
60	99

procedure was repeated for yeasts isolated from filling valves. A mixture containing 1ml of the stock culture solution and 19 mls of sterile distilled water in place of the chlorine was used as control.

Effect of Temperature on Yeast

McCartney bottles in duplicates were filled with 19mls of sterile distilled water. One ml of the sugar yeast stock culture solution was introduced into each of the McCartney bottles to make 20mls.

The bottles were heated at different temperature of 50, 60,70,80,90, and 100 degrees centigrade respectively for ten minutes in a water bath (Grant). The control bottles were not heated. Membrane filtration was done thereafter using Schaufus pottinger media. The same procedure was repeated for yeasts from filling valves.

Correlation Analysis

Similarities between sugar yeasts and filling valve yeast were analyzed using the correlation function of the Excel application in Microsoft 2000 computer software.

RESULTS AND DISCUSSION

Yeasts isolated from sugar and filling valves were cream colored, ovoid, raised, and intact and were between 3-10 microns in diameter. Budding was observed for some cells. Gram reaction showed it to be gram positive.

The stock solution plated on malt extract agar produced colonies similar to the ones described above. The control plate had growth of 98 cfu/20mls but no growth was observed at 60mg/ml for sugar and filling valve yeast. Also for all the samples no growth was observed after 24 hours. However yeast count was higher after 5 days for sugar yeasts than yeasts from filling valves at 10mg/ml concentration (Fig.1). Yeast count was also noticed to be higher for sugar yeasts than filling valve yeasts at 20mg/ml (Fig.2) 30mg/ml (Fig.3) and 40mg/ml (Fig.4).

At 50mg/ml (Fig.5) yeasts from filling valves had no growth while yeasts from sugar had a growth of 3 cfu/20ml after 5 days. It was found that yeast count decreased with increase in chlorine concentration. For instance at 10mg/ml, the highest yeast count was 56 cfu/20ml while at 50mg/ml the highest yeast count was 3 cfu/20ml.

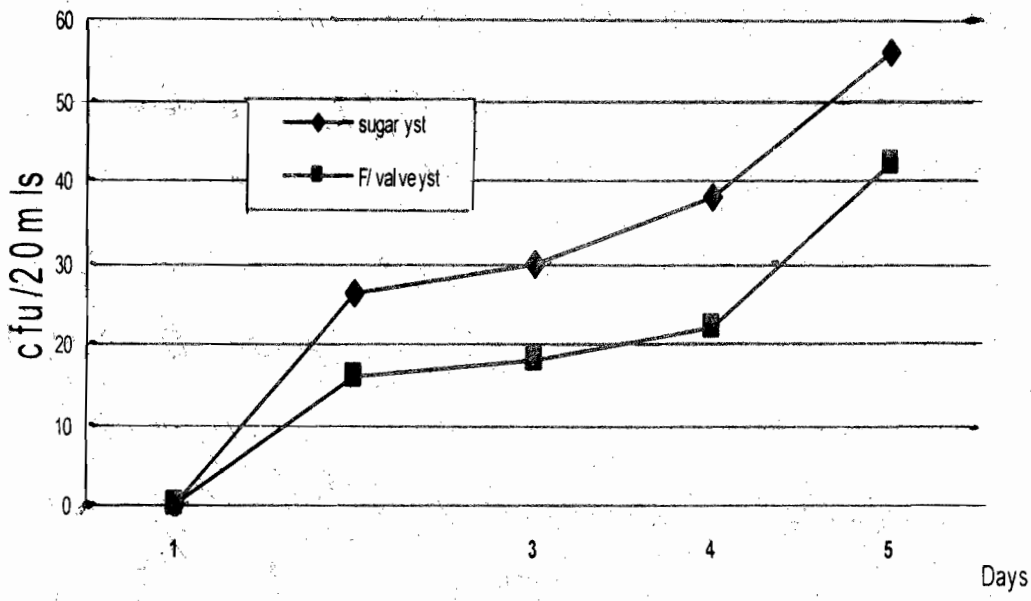

Fig.1Effect of chlorine on sugar and filling valve yeasts at 10mg/ml concentration.

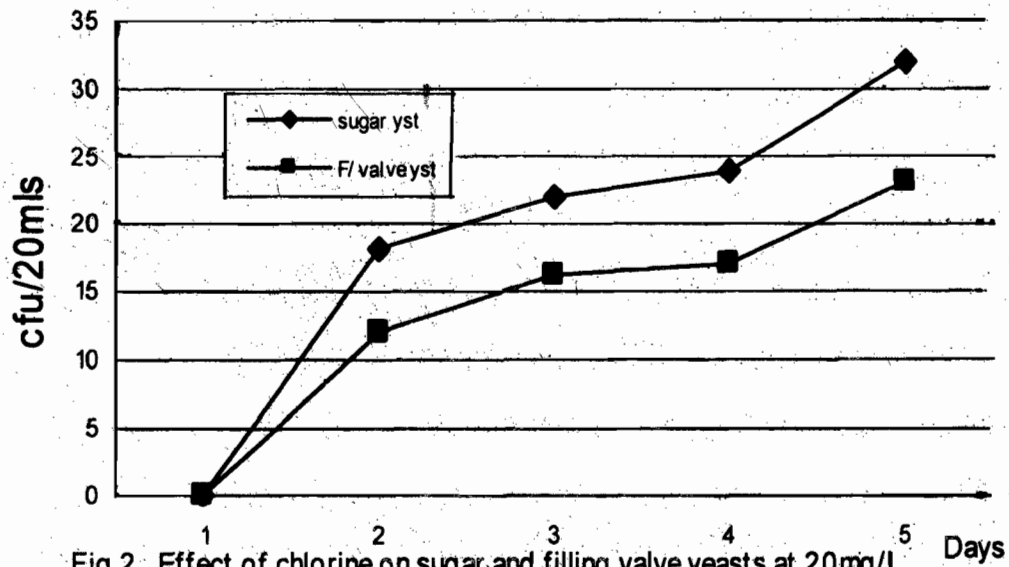

Fig.2. Effect of chlorine on sugar and filling valve yeasts at 20mg/l conentration.

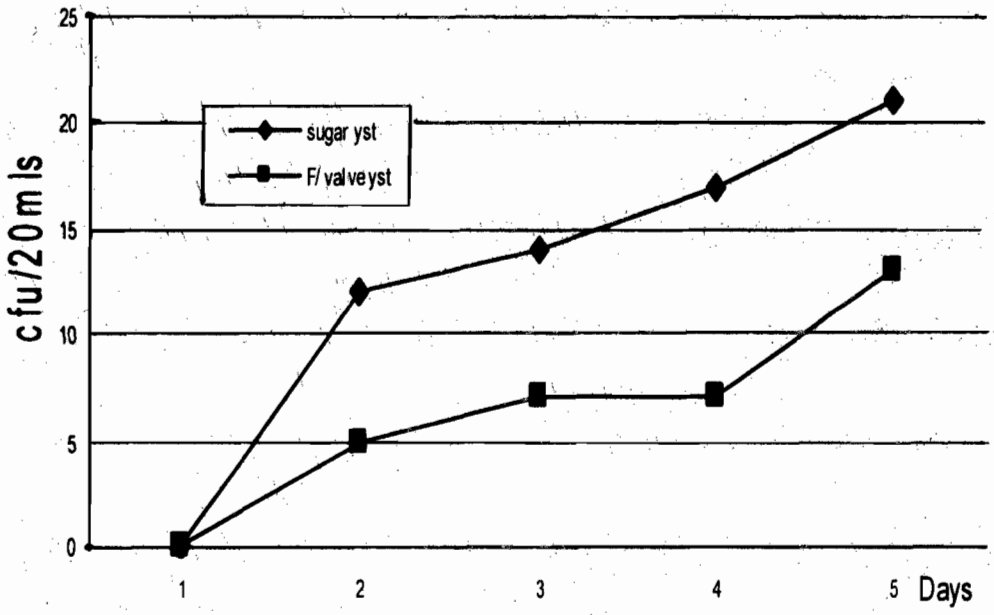

Fig.3. Effect of chlorine on sugar and filling valve yeasts at 30mg/l concentration.

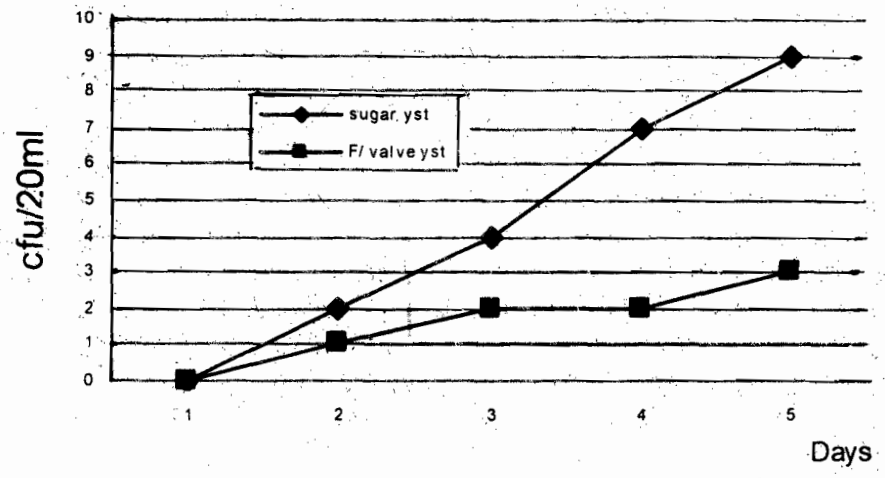

Fig.4. Effects of chlorine on sugar and filling valve yeast at 40mg/l concentration

The control had growth of 90 cfu/20mls while no growth was observed for both type of yeasts heated at 80, 90 and 100 degrees centigrade respectively. However, at 50°C growth was observed and found to be higher in sugar yeast than filling valve yeast (Fig.6). Sugar yeasts also had a higher count at 60°C (Fig.7). No growth was observed for filling valve yeast at 70°C while growth was seen for sugar yeasts. (Fig.8).

Table one shows the correlation analysis done for sugar and filling valves exposed to chlorine and heat. High correlation was observed with more similarity noticed in temperature reaction than that of chlorine.

The shape, size, color and budding observed show that the organisms isolated were yeasts. Also the gram positive reaction of the stained cells points out that dye in the cells was not decolorized. Gram positive cells retain the purple color after decolourization (Cheesebrough, 1985). Again Nwaiwu and Ibekwe (2002) isolated yeasts from filling valves while several workers have isolated yeasts from sugar (NBC, 2002, Delonix, 2004, Thrall, 2004).

Chlorine is inhibitory to the yeast cells since the highest yeast count was observed for the control samples which had no chlorine present.

The decrease in yeast count with increase in chlorine concentration and the contact time observed shows that time of exposure and higher chlorine concentrations were enough to inhibit the growth of yeasts isolated. Adequate time and concentration are necessary to allow hypochlorous acid to penetrate the cell walls and destroy the ability of yeast cells to form proteins (Wild, 1997 and Bartz, 1998). Also it has been reported that the ability of chlorine to reduce microbial count depends in part to contact time and concentration (Palin, 1983 and Carson *et al*, 1995).

For operations in Nigerian bottling company, the minimum inhibitory concentration is that concentration that would give yeast growth of less than 10cfu/20mls (Anonymous, 2002). Thus, 40mg/ml chlorine concentration is observed in this study to be the minimum inhibitory concentration for yeasts isolated from sugar and filling valves, since growth observed for both yeasts is less than 10cfu/20ml at that concentration (Fig.4).

The growth of sugar yeasts at 50mg/ml and lack of growth for filling valves indicate that the sugar yeasts have a higher chlorine demand. The higher chlorine demand by sugar yeasts is probably due to the source from which it was isolated. Sugar is an organic compound while filling valves are made of stainless material hence yeasts isolated from sugar had more access to organic material than yeasts isolated from filling valves. Palin (1983) reports that when chlorine is used as an inhibitory agent, the demand for it by organisms increases if such organisms are in contact with debris or organic material.

High yeast count obtained for both types of yeast at chlorine concentrations of 30mg/ml and below suggests that these concentrations are non-inhibitory. Thus any chlorine concentration within this range can be regarded as the non-inhibitory concentrations of sugar and filling valve yeast.

Lambert and Stratford (1999) described non inhibitory concentrations as those concentrations that would allow growth close to what is obtained during normal conditions.

The inability to observe growth at temperatures of 80° C and above shows that the yeasts isolated are sensitive to heat. The control samples which were not exposed to heat had high yeast count. Microorganisms would not survive if subjected to sufficient quantities of heat at sufficient intensity. This is because enzymes vital to life are irreversibly denatured (Jay, 1985). Several investigators have reported that yeast cells are damaged at temperatures of 80°C and above (Devinsky *et al.*, 1991, Anonymous, 2002 and Delonix, 2004).

Growth observed at 50°C show that yeasts isolated are mesophillic. Mesophillic organisms grow and proliferate at a temperature range of 20-50°C (Nester *et al.*, 1983). The observation of growth at 70°C for sugar yeasts and lack of it for filling valves yeast indicate that the molecules of the filling valves yeast reach their highest vibration energy before that of the sugar yeast. Temperature is a measure of vibration energy and if it is high enough an organism would die after some time (Devinsky *et al.*, 1985, 1991).

The high correlation coefficient observed for both types of yeast at certain temperatures and chlorine concentrations suggests that both yeasts are very similar and may be of the same specie.

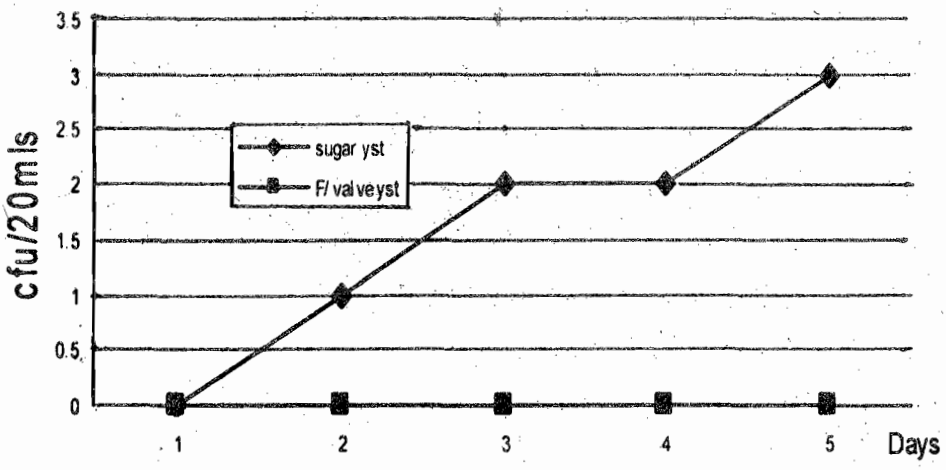

Fig.5. Effect of chlorine on sugar and filling valve yeast at 50mg/l concentration.

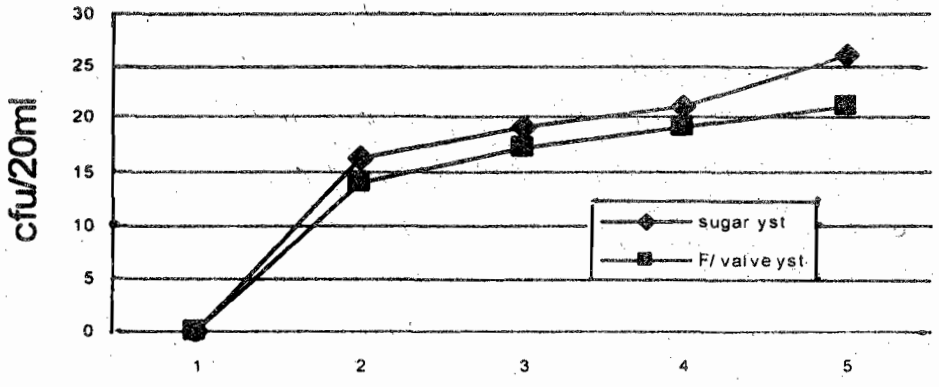

Fig.6. Growth of sugar yeasts and filling valve yeast heated to 50°C Days

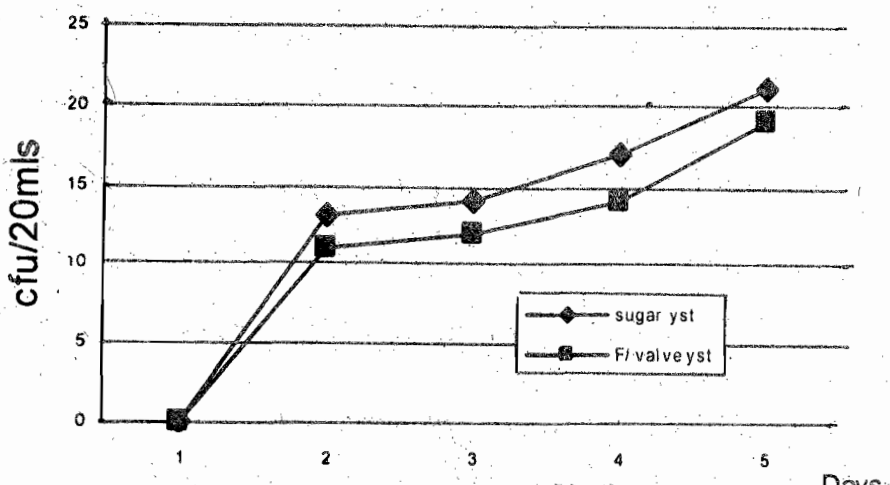

Fig.7. Growth of sugar and filling valve yeasts exposed to 60°C

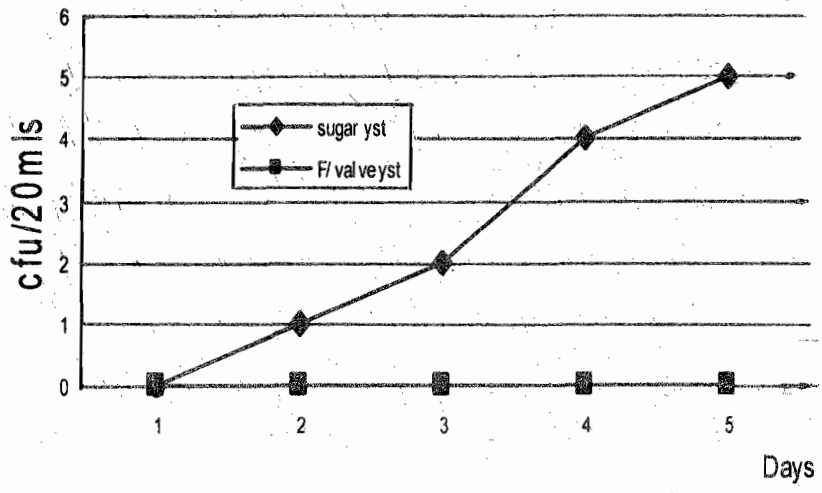

Fig.8. Growth of sugar and filling valve yeasts exposed to 70°C

In conclusion, high chlorine concentrations and temperatures can inhibit the growth of yeasts in a carbonated soft drink beverage production process. The optimum chlorine concentration for this study is 60mg/l, while the optimum temperature is 80°C. The high correlation between the sugar and filling valve yeast would require further studies to ascertain succession and population dynamics of the production process.

More rapid methods like analysis profile index and adenosine triphosphate bioluminescence would be ideal. Knowing the yeast content of the process would enable critical control points to be identified. Identification of critical control points is a key step in the implementation of hazard analysis and critical control points, a preventive programme that assures food safety (Nwaiwu and Ibekwe, 2003).

In these days of stringent regulatory control by Standards Organization of Nigeria (SON), National Agency for Food, Administration and Control (NAFDAC), carbonated soft drink manufacturers are expected to keep their environment yeast free. Hence identifying the areas of high yeast contamination potential and knowing the factors that can prevent yeasts from proliferating is a step in the right direction

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