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Prevalence and risk of heterotrophic microorganisms in a carbonated soft drink factory

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A farm to fork approach was used to establish the prevalence and risk of microorganisms in a carbonated soft drinks factory in south eastern Nigeria. Raw materials, intermediate and finished products were collected from 19 microbiological control points in a processing environment and analysed using membrane filtration over a 12 month period. Yeasts and other heterotrophic bacteria increased during the rainy season but there was no significant difference ($p>0.05$) in the proliferation of organisms among the analysed months. Although, organisms were not detected in finished products, there was 100% prevalence for yeasts and bacteria in the processing areas with high sugar activity. No mould, *Pseudomonas aeruginosa* or *Escherichia coli* were isolated. Overall, a 5 by 5 risk matrix showed that heterotrophic bacteria, yeasts or mould had low risk of reaching undesirable numbers. In addition to the prevalence investigation carried out in the processing environment, 864 properly stored bottles of the same brand of carbonated soft drink in trade were purchased from different commercial locations over 12 months and screened. No yeasts mould or coliform bacteria were isolated from the sampling carried out. In conclusion, heterotrophic bacteria and yeasts thrive more on equipment than intermediate or finished products in the process environment studied and the risks of product spoilage or people getting ill was generally low and may remain so, if good manufacturing and proper after sales handling of the products are observed. Study highlights the need to focus on areas of high sugar activity, to control undesirable organisms and further work is required to establish how microorganisms survive and form biofilms on bottling equipment after sanitation.

Key words: Heterotrophic, yeasts, bacteria, prevalence, carbonated soft drinks, risk, biofilms, bottling equipment.

INTRODUCTION

Non-alcoholic carbonated soft drinks (CSD) are consumed in all corners of the globe and are served in most social functions. The preference of consumers for a particular soft drink is greatly influenced by the beverage's specific

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attributes (Redondo et al., 2014). The drink may be up to 98% water and may contain preservatives and carbon dioxide to prevent the proliferation of microorganisms (Juvonen et al., 2011). According to Kregiel (2015), microbial contamination of soft drinks may originate from the raw materials, factory environment, microbiological state of the equipment and lack of hygiene. Filamentous fungal and bacterial pathogens do not pose any risk due to the presence of carbon dioxide, low pH and preservatives but yeasts and aciduric bacteria can survive and cause spoilage (Azeredo et al., 2016).

In the developed world, high microbial load in soft drinks is rare and hardly reported possibly due to strict compliance with hazard analysis and critical control point programs (HACCP) running in processing plants. However, in the developing world some studies have shown that carbonated soft drinks are loaded with bacteria. Akond et al. (2009) found that carbonated soft drinks commercially available in Bangladesh contained coliform bacteria even though all samples were under the permissible limit for heterotrophic plate counts. Heterotrophic microorganisms require only carbon for energy and they include most bacteria found in the food processing environment especially *Escherichia coli*, yeasts, and mould (Health Canada, 2012). Heterotrophic count is not a pathogen indicator but it is used for general detection of any form of contamination (Amanidaz et al., 2015). The microbial limits for water and other foods are normally set by World Health Organisation (WHO) or health authorities of a country after several validation trials in the laboratory.

Prevalence of microorganisms in a food processing environment is aided by the formation of biofilms. Biofilms have been described as accumulated mass of microorganisms and their extracellular matrix on a solid surface (O'Toole et al., 2000).The process of adhesion to surfaces includes a reversible phase and an irreversible molecular phase (Donlan and Costerton, 2002). Acccording to Winkelströter et al. (2014) the biological cycle of biofilms depends on the organism and includes developmental phases such as initial attachment, maturation, maintenance, and dispersal. It is generally known that biofilms are difficult to remove from a food processing facility and may remain in the environment for a long period due to resistance to sanitizing agents (Chmielewski and Frank, 2003). Biofilm formation is a concern for the food industry because the colonization of food processing surfaces can lead to contamination of products and cause food poisoning or spoilage (Olszewska, 2013). This can cause huge economic losses to members of the public and the factory involved.

Contaminants and heavy metal assessment of commercial carbonated soft drinks samples in Nigeria have been carried out (Engwa et al., 2015) but the microbial profile has not been extensively reported. From literature search, the reports of analysis for process environment where carbonated soft drinks are produced in Nigeria are rare. Therefore, the main aim of this study was to establish the source, frequency and level of heterotrophic microbial contamination at different points within a carbonated soft drink bottling process. Also, the risk of contamination within the process environment and the prevalence of microorganisms in commercial products were evaluated.

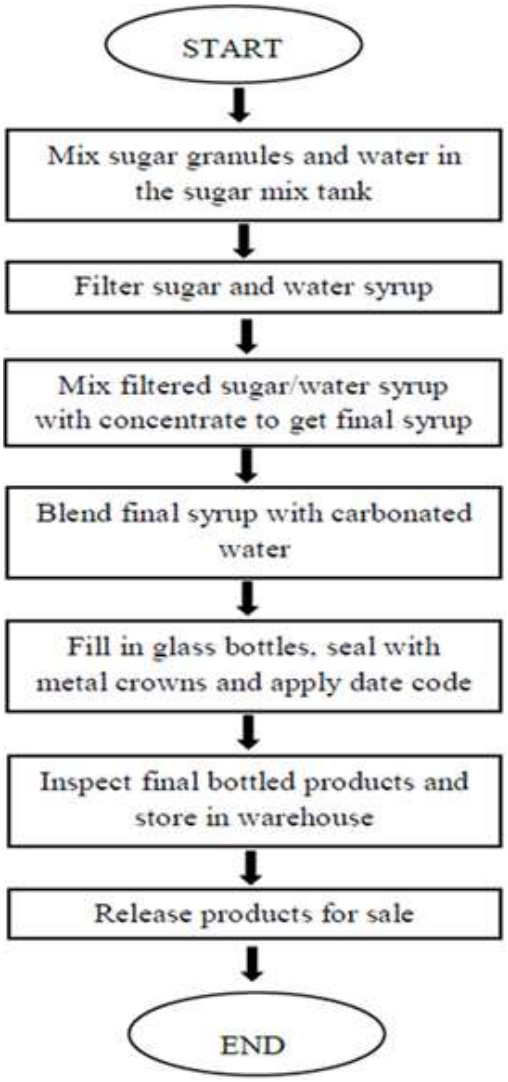

Figure 1. Flow chart showing different steps in the carbonated soft drink production process.

MATERIALS AND METHODS

Carbonated soft drink production process

Study was carried out in a CSD bottling facility in south east Nigeria. The production process of the carbonated beverage studied is shown in Figure 1. The main raw materials used are treated water, granulated sugar, carbon dioxide and beverage concentrate. Granulated sugar is mixed with water to get simple syrup after which

the simple syrup is filtered and mixed with concentrate to get more viscous final syrup. The final syrup is then blended with carbonated water and filled in washed returnable glass bottles with the aid of vent tubes after which, a final product inspection is carried out before warehouse storage. Products are sent to shops and then released for sale. A microbiological monitoring program is in place for sampling from several points. The microbiological program focuses on areas of microbial activity or absence of activity and microbial identification to genus level rather than classical multiple step identification are performed.

Sample collection

All the 19 sampling locations used covered the raw materials, sugar and treated water as well as intermediate products (simple and final syrup). They were sampled 3 times a month on different days over a 12 month period. Blended beverage and carbonated water were collected from the blender while bottled products were collected from the production line after filling. Random samples (three each) were collected at first, second and third turns of the filler in the start of the production run. Samples were also collected from the warehouse after 5 days storage. Cotton wool swab samples in triplicate were collected from processing equipment namely, simple and final syrup tank, polishing filter tank, blender and sugar mixing tank. Swabbing was carried out with a stainless steel rectangular template (8 x 5 cm). Filler swab was carried out on the vent tube which is a product contact component in the filler by swabbing 5 times for each test. The internal surface of the neck of washed bottles was also swabbed (10 circular strokes per bottle).

Swabs were broken off into 100 mL of 0.9% sodium chloride (NaCl) in sterile water contained in 100 mL conical flasks for bacteria count, 20 mL for yeast and mould count in universal bottles and then allowed to stand for 3 h before membrane filtration was carried out. Sugar granules were collected at random from sugar bags with a sterile spatula, during production of simple syrup with Whirl-Pak sampling bags (Nasco, Wisconsin, USA). A mixture was obtained by mixing 20 g of sugar with 200 mL of 0.9% NaCl in sterile water using 250 mL conical flasks. Liquid samples from water and all other intermediate and finished products were collected with Whirl-Pak sampling bags and analysed within 1 h after collection. In all liquid samples three replicates of 100 mL were used for bacteria count whereas 20 mL replicates were used for yeasts and mould analysis. For analysis of commercial CSD samples, 3 cases consisting of 24 bottles each were purchased from reputable commercial shops monthly and screened for the presence of heterotrophic microorganisms.

Prevalence and heterotrophic counts

Industrial standard membrane filtration (Hallasa and Monisa, 2015) was used to analyse factory and commercial samples. Filtration was carried out with a sterile multi-branched stainless steel manifold and filter holder system with different nutrient pad media sets (Sartorius, Göttingen, Germany) according to manufacturer's instructions. The nutrient pad media sets included tryptone glucose extract media for total bacteria count, tergitol triphenyltetrazolium chloride media pad for coliforms and enterobacteria, centrimide nutrient pad for *Pseudomonas Spp*. and other non-faecal pathogenic bacteria and Schaufus Pottinger nutrient pad for yeasts and mould. The membrane filter was attached to a nutrient pad after filteration and incubated at 25°C for 5 days for yeasts and mould and 37°C for 24 h for bacteria. Membrane filters of 0.65 µm was used for yeasts and mould whereas 0.45 µm membrane filter was used for bacteria.

Incubation of cells on centrimide media was carried out at 42°C for 48 h. Colonies that emerged were counted as colony forming units (CFU) per 100 mL or 20 mL of membrane filtration sample.

The percentage prevalence was calculated from the number of times the organism occurred in all samples (108 tested per sample point), of a sample point tested during the 12 month study.

Risk analysis

A 5 by 5 risk matrix (Cox, 2009) was used to estimate risk from likelihood (L) and severity or consequence (C) of counts obtained. Consequence or severity was assigned to bacteria and yeast counts based on where the organism occurred (Table 1) and Risk (R) of contamination was calculated by multiplying the values of likelihood and consequence (R=L x C). It is common to use broad categories to capture impact in a matrix (UN, 2012) and the risk scores may overlap (ISO, 2009).

In this study, risk scores in the range of 1 to 8, 9 to15 and 16 to 25 were regarded as low, medium and high risk, respectively as previously described (RAO, 2016). The risk rating used the, factory's stricter counting standard of 25 CFU/100 mL for heterotrophic bacteria as a reference point rather than generally known 500 CFU/mL (Health Canada, 2012). For yeasts and mould, Nigeria Industrial Standards (SON, 1992) stricter limit of 1 CFU/mL was used as a reference point instead of the maximum 5 CFU/mL (Sartorius, 2016) advised by the manufacturers of the membrane filtration kit.

Statistical analysis

Means, correlation and analysis of variance were determined using Minitab 17 software (Minitab Inc., PA, USA). Statistical significance cut off was set at $p < 0.05$.

RESULTS AND DISCUSSION

In the factory studied, any growth trend is monitored and is relied upon for corrective actions. There is less emphasis on statistical significance of microbial counts mainly because statistical significance does not necessarily establish practical significance (Murtaugh, 2014). The isolation of heterotrophic bacteria (Table 2a) and yeasts (Table 2b) showed that, the highest prevalence (100%) for both organisms was detected from syrup mix and simple syrup tank. None of these groups of organisms were detected in the finished products that contained carbon dioxide and prevalence was highest (100%) in areas of high sugar activity.

In Nigeria, the rainy season is between March and October, and the sales of CSD plummet, when sales go down, production is scaled down in the factory and the equipment remains idle for long periods. According to Watkinson (2008), a variety of microorganisms may remain and slowly accumulate on processing equipment even when the equipment appear clean and while processing equipment is idle, significant number of bacteria may develop even though the equipment has been cleaned and disinfected. There was an increase (up to 50%; Table 2) in count for both groups of organisms, during the rainy season months of March to September but no significant difference ($p > 0.05$) was observed in the count of organisms between all months evaluated. It

Table 1. Categories used for risk assessment of microbial contamination.

Assessment		Score
(i) Likelihood (L)		
Yeast (cfu/20 ml)	**Bacteria count (cfu/100 ml)**	
0-4	0-5	1
5-8	6-10	2
9-12	11-15	3
13-16	16-20	4
17-20 or more	21 or more	5
(ii) Consequence or severity (C)		
Occurrence in:		
Raw material		1
Equipment		2
Intermediate product e.g. syrup		3
Blended beverage before bottling		4
Bottled beverage		5
(iii) Risk (R) = Likelihood (L) x Consequence or severity (C)*		

*Cox (2009).

Table 2a. Average heterotrophic bacteria count and percentage prevalence (**%)** in different samples over 12 months.

Sample source	Counts* Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Prevalence (%)
Sugar mixing tank swab	80±2.0	40±1.2	65±.0.9	69±0.2	42±0.6	60±0.1	80±0.3	90±0.2	77±1.3	75±1.1	63±0.2	78±0.3	100
Simple syrup tank swab	132±2.5	126±1.8	120±0.4	110±0.3	133±1.4	150±0.1	165±0.1	185±0.4	131±1.1	128±1.2	114±1.5	122±0.5	100
Final syrup tank swab	14±2.0	0	0	0	0	0	0	0	0	0	0	0	11
Beverage Blender swab	5±0.6	1±0.0	1±0.1	2±0.1	0	0	2±0.0	3±0.3	1±0.0	0	0	0	58
Polishing filter tank swab	10±1.0	9±0.4	2±0.2	1±0.0	0	2±0.0	5±0.1	6±1.2	3±0.1	2±0.0	1±0.0	1±0.0	94
Filler swab	6±1.0	15±0.8	22±0.6	25±0.4	15±0.1	10±0.2	26±1.5	22±1.4	18±0.2	12±0.2	9±0.4	11±0.2	100
Washed glass bottles swab	0	0	1±0.0	0	5±1.5	1±0.0	2±0.0	2±0.0	1±0.0	1±0.0	1±0.0	0	67
Treated Water	0	0	0	0	0	0	0	0	0	0	0	0	0
Filler Rinse water	2±1.0	3±0.1	1±0.0	1±0.0	9±0.5	7±0.1	10±0.5	11±0.2	14±0.5	16±0.4	2±0.0	6±0.1	92
Sugar crystals	0	0	0	0	0	0	0	0	0	0	0	0	0
Simple syrup before filteration	22±1.5	30±1.1	22±0.1	34±0.3	28±0.1	35±0.2	56±0.5	44±0.4	32±1.3	38±0.5	28±1.4	22±1.2	100

Table 2a. Contd.

Simple syrup after filteration	18±1.5	14±0.5	12±0.2	17±0.6	20±0.4	22±0.2	38±0.1	19±0.3	12±1.2	10±0.3	15±1.1	10±0.5	100
Final syrup	2±0.0	0	0	0	0	0	0	0	0	0	0	0	8
Carbonated water	0	0	0	0	0	0	0	0	0	0	0	0	0
Blended beverage before bottling	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, Ist turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, 2nd turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, 3rd turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Warehouse finished product	0	0	0	0	0	0	0	0	0	0	0	0	0

*Liquid samples = CFU 100/mL; Sugar granules = CFU 20/g; Swab samples = CFU/40cm^2; Anova = p>0.05 between months.

Table 2b. Average heterotrophic yeast count and percentage prevalence (**%)** in different samples over 12 months.

Sample source	Counts* Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Prevalence (%)
Sugar mixing tank swab	18±1.1	14±0.4	56±0.1	66±0.2	52±0.1	60±0.3	77±0.6	64±1.1	57±1.3	40±0.2	33±0.2	29±0.2	100
Simple syrup tank swab	12±1.5	4±1.7	66±0.5	74±1.9	72±0.5	45±0.1	79±0.2	86±0.2	62±0.6	28±0.4	22±0.2	28±1.2	100
Final syrup tank swab	5±1.0	0	0	0	0	0	0	0	0	0	0	0	25
Beverage Blender swab	5±1.0	0	0	0	0	0	0	0	0	0	0	0	8
Polishing filter tank swab	8±0.5	0	0	0	0	2±0.0	2±0.2	1±0.0	0	0	0	0	33
Filler swab	6±0.6	0	0	0	0	0	0	1±0.0	0	0	0	0	17
Washed glass bottles swab	0	0	1±0.0	0	5±0.3	1±0.0	2±0.0	2±0.0	1±0.0	1±0.0	1±0.0	0	67
Treated Water	0	0	0	0	0	0	0	0	0	0	0	0	0
Filler Rinse water	1±0.0	2±1.0	1±0.0	0	6±0.3	5±0.2	8±0.4	6±0.5	6±0.2	10±0.2	1±0.0	4±0.1	94
Sugar crystals	0	0	0	0	0	0	0	0	0	0	0	0	0
Simple syrup before filteration	14±2.	17±0.6	12±0.4	24±0.3	21±0.1	23±0.4	22±0.4	27±1.4	16±1.1	21±0.4	12±0.5	14±0.3	100
Simple syrup after filteration	8±1.2	5±1.0	4±0.1	17±0.5	10±0.1	15±0.3	9±0.5	9±0.2	5±0.3	10±1.1	8±0.1	5±0.1	100
Final syrup	0	0	0	0	0	0	0	0	0	0	0	0	0
Carbonated water	0	0	0	0	0	0	0	0	0	0	0	0	0
Blended beverage before bottling	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, Ist turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, 2nd turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Finished product, 3rd turn of filler	0	0	0	0	0	0	0	0	0	0	0	0	0
Warehouse finished product	0	0	0	0	0	0	0	0	0	0	0	0	0

*Liquid samples = CFU 100/mL; Sugar granules = CFU 20/g; Swab samples = CFU/40 cm^2;Anova = p>0.05 between months.

has been explained (Zeraik and Nitschke, 2012) that, environmental conditions can influence the complex process of bacterial adhesion to inert surfaces. Hence, the higher counts between March and September among the months analysed for microbial count (Table 2) may be due to, the prolonged period that the bottling equipment was kept idle which allowed microorganisms to proliferate. Another contributing factor could be the seasonal differences in temperature and water activity experienced during rainy or dry season because, temperature and water activity affects growth rate of bacteria (Medveďová et al., 2009).

In the high sugar activity areas, correlation analysis showed that there were similarities in bacteria ($r=0.73$; $p<0.05$) and yeast ($r=0.72$; $p<0.05$) growth patterns of samples from simple syrup before and after filtration (Table 2). Counts of swab samples from sugar mix tank and the simple syrup tank showed low similarities for bacteria ($r=0.41$; $p<0.05$) while the highest similarity was observed for yeast ($r=0.93$; $p<0.05$). This indicates that, microbial growth persisted and remained consistent in intermediate products and on bottling equipment. Lawlor, et al. (2009) has noted that yeasts and aciduric bacteria can survive and grow in the physical and chemical conditions of a CSD and its bottling equipment. Furthermore, persistence of microrganisms observed in this study is common in food processing environment and may be attributed to biofilm persistence (Coughlan et al., 2016). The layers of biofilms matrix contain sub-populations of bacteria and these contaminations could be enhanced, if more than one microbial species is responsible for the formation of the biofilm matrix (Singh et al., 2012). This has been shown by Roder et al. (2015) after co-cultures of bacteria isolated from a food processing facility, were used to show enhanced biofilm formation. The interspecies interactions observed in this study and the nature of biofilm formed will need to be investigated.

No mould, *Pseudomonas aeruginosa* or *E. coli* were isolated during the 12 months period the study was carried out. It was found that in filler swabs and washed glass samples, bacteria was more prevalent (Table 2). All the products purchased monthly over a 12 month period from different trade locations did not show any growth on the nutrient pads used. Soft drink producers have the problem of preventing contamination in the manufacturing process and if microorganisms are allowed to proliferate, they can distort the taste of soft drinks and possibly cause illness. The lack of growth in sugar granule samples and growth in the sugar mix tank indicates that, equipment where sugar was processed is prone to contamination. Results (Table 2) indicated that filtration of the simple syrup reduced bacteria and yeast count. Theoretically, it would be beneficial to repeat the filtration process to reduce number of organisms further during production, but this will not be practicable because of the time it will take to filter large volumes of syrup usually prepared. The prevalence (100%) of bacteria and yeasts in areas of high sugar concentration is most likely, due to the fact that the organisms had access to nutrient and growth requirements (Sperber, 2009), in areas where sugar was used regularly. The lack of growth in samples with carbon dioxide was most likely due to low oxygen availability, under the fungistatic and bacteriostatic environment caused by the carbon dioxide inherent in the finished products (Wareing and Davenport, 2007). It may also be due to metabiosis because it has been shown that, bacteria and fungi can form a range of physical associations that depend on various modes of communication for their development and function (Frey-Klett et al., 2011).

The filling machine sanitation status was shown by the filler rinse water and the maximum yeast count obtained (Table 2b) was slightly higher than counts obtained previously (Nwaiwu and Ibekwe, 2006), when filler components were evaluated for their contamination potential. The rinse water showed the true sanitation status of the filler prior to the beginning of beverage production because it flowed over filler components like vent tubes in the filler before it was captured for analysis. Yeasts and bacteria detected in the rinse water indicates presence of microorganisms in the filler but, the number was not sufficient to overcome the carbon dioxide barrier in the finished products which was evidenced by lack of growth in all nutrient growth pads used for testing finished products. The commercial samples were free of mould, yeasts and bacteria because the carbon dioxide and preservatives in the product were able to prevent the organisms, inherent in the product from getting to detectable thresholds. This suggests that detection may only be possible with initial enrichment of the CSD sample and longer incubation after plating. In line with this study, commercial carbonated water has been analysed elsewhere (Saleh et al., 2008) and no bacteria were detected in the water. In another study by Aljaloud (2016) on commercial non- alcoholic energy drinks, it was found that most of the carbonated energy drinks analysed did not contain harmful bacteria and total bacterial counts for most of the samples were less than 1 log CFU/mL). As pointed out by Ashurst (2009), the main factor in determining shelf life for carbonated beverages, is the retention of carbon-dioxide in finished products.

Efiuvweuwere and Chinyere (2001) found that incidence of *Bacillus* spp. in carbonated soft drink held in open-air was, remarkably increased for samples stored at over 30°C by approximately 103% when they compared trade samples stored at different temperatures. In contrast to lack of microbial growth for commercial samples purchased in this study, Oranusi et al. (1994) detected contaminants in 50% of samples analysed in an investigation while the other half showed no growth. In another study, Okpalugo et al. (2008) found that only 35% of samples were without growth and concluded that there were a lot of differences in the microbial content of

Table 3. Risk of contamination rating for bacteria and yeasts based on likelihood and consequence (severity).

Sample source	Likelihood		Consequence	Risk score		Risk rating	
	Bacteria	Yeast		Bacteria	Yeast	Bacteria	Yeast
Sugar mixing tank swab	5	5	2	10	10	Medium	Medium
Simple syrup tank swab	5	5	2	10	10	Medium	Medium
Final syrup tank swab	1	1	2	2	2	Low	Low
Beverage Blender swab	1	1	2	2	2	Low	Low
Polishing filter tank swab	1	1	2	2	2	Low	Low
Filler swab	1	1	2	2	2	Low	Low
Washed glass bottles swab	1	1	2	2	2	Low	Low
Treated Water	1	1	1	1	1	Low	Low
Filler Rinse water	1	2	3	3	6	Low	Low
Sugar crystals	1	1	1	1	1	Low	Low
Simple syrup before filteration	5	5	3	15	15	Medium	Medium
Simple syrup after filteration	4	2	3	15	6	Medium	Low
Final syrup	1	1	3	3	3	Low	Low
Carbonated water	1	1	3	3	3	Low	Low
Blended beverage before bottling	1	1	4	4	4	Low	Low
Finished product, Ist turn of filler	1	1	4	4	4	Low	Low
Finished product, 2nd turn of filler	1	1	4	4	4	Low	Low
Finished product, 3rd turn of filler	1	1	4	4	4	Low	Low
Warehouse finished product	1	1	5	4	4	Low	Low

Risk rating based on risk scores; 1-8 = low risk; 9-15 = medium risk; 16-25 = high risk.

different production batches of CSD. In Nigeria, it is not uncommon to see CSD displayed in open air for sale in direct sunlight. Also retailers sometimes exhibit poor handling of products by stacking cases roughly and cause the metallic crown corks of the product to shift. In some cases, products with compromised crown cork may lose carbon dioxide and facilitate the growth of yeast and bacteria. None of these undesirable practices were encountered in this study and may have contributed in part to the non-detection of microbial growth, in commercial samples. A link between environmental contamination and prevalence of microorganisms has been suggested (Shanker et al., 2012), hence further studies need to be carried out, taking into consideration the different environment where CSDs are sold.

The analytical method employed in evaluation of soft drinks for microbial content by other workers and the source of samples could also explain the differences in the results of the analysis of commercial samples in this study. In the aforementioned studies, the retail outlets where the samples, were purchased appear to be small outlets where handling and good warehouse practices may not be standard and products are left in open air. If a product becomes compromised for any reason, bacteria can grow within a few hours and cause increased detection during sampling. This has been demonstrated by Park and Chen (2009) who found that, soft drinks sampled after the 4h holding period had relatively higher counts than those sampled initially.

Microbial risk assessment can be used to manage the risk posed by food pathogens (FAO/WHO, 2009). According to Cox (2008) when risk matrixes are used to evaluate risks, categorization may require subjective interpretations and careful explanations should be given to reduce subjectivity in the matrix used for risk quantification. The limits adopted by the factory based on local regulation (SON, 1992) were used as a reference point and the risk rating (Table 3) showed that in most sample points, there was low risk of contamination. In agreement with the prevalence counts (Table 2), only areas with high sugar processing showed a medium risk of contamination. The quantification of risks with the matrix employed is not absolute and the emphasis was on the quality control perspective brought to the study in line with HACCP principles. As suggested by Pickering and Cowley (2010), a hazard management process may lead to timely decision making and a better use of resources. In this case, more attention should be paid to areas of high sugar activity where most of the bacteria and yeast occurred.

The factory studied uses a stricter limit for occurrence of microorganisms after sampling, to ensure that corrective actions are taken before the organisms proliferate and easily reach detectable thresholds in the final product. This approach ensures that they stay within the local, national and international permissible limits (500 CFU/mL). In addition to the growth retardation effect of carbon dioxide on microorganisms, most CSD

manufacturers around the world maintain high sanitation and good manufacturing practices. This may be why in spite of billions CSD served daily around the world, it is rare to see reports of sporadic outbreak of microbial infection after consumption. Further work which takes into consideration the equipment design and factory personnel hygiene practices, while exploring how yeast and bacteria survive on the equipment during idle periods, will contribute in maintaining low risk of contamination in the process environment.

Conclusion

Overall, the heterotrophic count especially for bacteria was far less than known limits (500 CFU/mL) which suggest good sanitation practices and low risks of product spoilage. A definitive finding is that, heterotrophic bacteria and yeasts thrived more on equipment than intermediate or finished products in the process environment analysed.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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