Sudan University of Science and Technology

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Effect of Quality System on the Microbiological Quality of Sudanese Carbonated Soft Drinks

أثر نظام الجودة على الجودة الميكروبيولوجية للمشروبات الغازية السودانية

BY

Eltahir Ahmed Mohamed Ahmed Hassan

B.Sc (Honours), Department of Food Science and Technology, Sudan University of Science and Technology, College of Agricultural Studies (2010)

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Supervisor

Dr. Mahdi Abass Saad Shakak

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Dedication

I dedicate this thesis to my family for nursing me with affection and love and their dedicated partnership for success in my life

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All praise is due toAllah, the Lord of the Worlds.

The Beneficent, the Merciful.

Master of the Day of Judgment.

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Abstract

Three brands of non-alcoholic carbonated soft drinks (CSDs) from three plants in Khartoum, Sudan which were named A, B and C; were examined for their microbiological quality. Plants were selected according to their quality system 'A being the highest C being the lowest'. Samples were analyzed for (TBC); Yeast and Mold, coliform bacteria and acid tolerant microorganisms using membrane filtration method.

Carbonated soft drinks from Plant C were the worst in microbiological qualitywith results in colony forming units ranging from 26 to 39 cfu/100 ml. for TBC, 9 to 34cfu/100 ml. for yeast, 1 to 5 cfu/100 ml. for mold and 30 to 59 cfu/100 ml. for acid tolerant microbes. These results were a reflection of the poor quality system of plant C.

Results from plant A and B showed Nil to 1 cfu/100 ml. for yeast, mold and acid tolerant bacteria. Nil to 3cfu/100 ml. for total bacterial count.

The study concluded to the significance of applying quality systems in the Sudanese soft drinks industry. Carbonated soft drinks plants A and B which were having quality systems such as ISO 22000:2005 and good manufacturing practice; were better in microbiological quality than the plant operating with no solid quality system.

Samples taken from the three plants generally didn't comply with The Sudanese standard SSMO (250/2007) even though results were significantly better in plants A and B as shown by the statistical analysis of the microbiological results. Nevertheless, absence of coliform bacteria in samples taken from the three plants was the only criterion that was met in SSMO (250/2007).

ملخص الأطروحة

تمت دراسة ثلاثة منتجات لمشروبات غازية غير كحولية منتجة من ثلاث مصانع مختلفة في مدينة الخرطوم, السودان. وذلك لمعرفة جودتها المايكروبية.تم اختيار المصانع حسب نظام الجودة المطبق فيها و سميت ب B,A و C بحيث مصنع (A) هو الأفضل في نظام الجودة والمصنع (C) هو الأسوأ في نظام الجودة. تم تحليل ودراسة العينات من حيث معرفة العد المايكروبي الكلي للعينات, الخمائر, الأعفان, باكتيريا القولون والمايكروبات المحبه للحموضة باستعمال طريقة الفلترة المايكروبية.

كانت نتائج العينات المتحصل عليها من المصنع (C) الأسوأ في الجودة المايكروبية بأرقام عد مايكروبي تتراوح بين 26 و 100/cfu39 مل للعد المايكروبي الكلي, 9 و34 100/cfu مل للخمائر, 1 و5 100/cfu مل للأعفان, 30 و 100/cfu مل 59 للمايكروبات المحبة للحموضة. نتائج العينات المتحصل عليها من المصنع (C) كانت إنعكاس مباشر لضعف و عدم تطبيق المصنع (C) لنظام جودة واضح أثناء فترة الدراسة.

نتائج العينات المتحصل عليها من المصانع (A) و(B) كانت مابين صفر و 1 100/cfu مل للخمائر, الأعفان والمايكروبات المحبة للحموضة. و كانت مابين صفر و 3 100/cfu مل للعد المايكروبي اللكلي.

خلصت الدراسة إلى أهمية تطبيق أنظمة الجودة في صناعة المشروبات الغازية السودانية. المصانع (A) و(B) والتي تطبق انظمة الجودة ISO 22000:2005 و ممارسات التصنيع الجيدة كانت وبصورة فارقة أفضل في جودتها المايكروبية من المصنع (C) الغير مطبق لنظام جودة كما أظهرته نتائج هذه الدراسة.

أظهرت نتائج التحليل المايكروبي للمشروبات الغازية المأخوذة من المصانع الثلاث (A),(B) و(C) عدم مطابقة للمواصفة القياسية السودانية الخاصة بالمشروبات الغازية رقم م.ق.س (250/2007)بالنسبة لجودتها المايكروبية. على الرغم من أن نتائج التحليل المايكروبي كانت أفضل بالنسبة للمصنعين A وB كما اظهرته نتائج التحليل الإحصائي لنتائج التحليل المايكروبي في هذة الدراسة. الجدير بالذكر هو خلو المشروبات الغازية من المصانع الثلاث من باكتيريا القولون.

CHAPTER ONE

INTRODUCTION

Carbonated soft drinks have been an essential part of the contemporary diet. The market for carbonated beverages has grown dramatically in most countries, for example, by 128% in the UK since 1984 as stated by Steen and Ashurst (2006). This growth has required changes in the way factories are run and operated to meet new legal, statutory and hygienic requirements. Soft drinks are now classified as food products and are produced under stringent hygiene conditions (Steen and Ashurst, 2006). Same case applies to The Sudan where consumption increased drastically in the last decade and more than eight soft drinks factories operate in the capital Khartoum alone.

The last point raises a concern over the level of Sudanese manufacturing conditions; since some of these manufacturers' operate without quality systems and microbiological testing to their product; bearing in mind that it is absolutely a fundamental requirement of any food process that the food produced should be safe for human consumption (Brennan and Grandison, 2011).

Health benefits of carbonated soft drinks such as, they are being usually absorbed more readily than water (because of their osmolality), can replace lost salts and energy quickly and are rapidly thirst quenching (Steen and Ashurst, 2006). Making it of high significance in hot tropical countries like the Sudan; where good hydration is essential for human beings

In the 21st century, food safety issues have as high a priority and significance as they did over 100 years ago (Schmidt and Rodrick, 2006).

Quality systems contributed positively to the integrity of food including carbonated soft drinks. Quality systems such as ISO 22000:2005, HACCP,

Good Manufacturing Practice, Good Hygiene Practice, and Good Agricultural Practice and so on have been developed and created to control food hazards in food production (Jeremy, 2006).

The aim of this dissertation is to study the argument of whether there are differences in microbiological profile of soft drinks produced using different quality philosophies which are Good Manufacturing Practice (GMP), (ISO 22000:2005) and a plant with no quality philosophy all; that is in Khartoum, Sudan.

The objectives of this study were to:

1. Study the microbial quality (TBC, total yeast and mold, coliform bacteria and acid tolerant microbes) of carbonated soft drinks sampled from three different plants.
2. Verify the HACCP plan in ISO 22000:2005 in plant A.
3. Determine which quality system gives the best microbiological results.

CHAPTER TWO

LITERATURE REVIEW

2.1 Carbonated soft drinks (CSDs)

2.1.1 Definition

What are carbonated soft drinks? There is no single definition available but it is generally accepted that they are sweetened water-based beverages, usually with a balancing acidity and are flavored (Ashurst, 2005).

Carbonated soft drinks are also defined as bottled or canned non-alcoholic, carbonated, flavored beverages that are usually served cold (Hoffmann *et al*., 1997).

The term ‘soft drink’ applies to beverages containing flavorings and/or fruit juices together with other constituents of technological or nutritional value designed to enhance the appearance and stability of the product and to ensure its organoleptic properties remain intact during a reasonable shelf life. These factors are taken into consideration in all development work, and in order to meet current stringent quality and legislative controls a new beverage is subjected to extensive trials to assess the suitability and performance of all components in its makeup. It becomes essential to arrive at the correct ingredient formulation to achieve a reproducible product (Ashurst, 2005).

Sudanese standard and metrology organization (SSMO,2007) defines carbonated soft drinks as drinks prepared by pressurizing carbon dioxide in water then adding sucrose and any other sweeteners approved by SSMO to it.

SSMO (2007) reported that, carbonated soft drinks are classified as the following:

Natural carbonated soft drinks: Drinks prepared by pressurized carbon dioxide in water then added natural fruit juice and sucrose and or any other sweeteners approved by SSMO to it.

Synthetic carbonated soft drinks: Drinks prepared by pressurizing carbon dioxide in water; a synthetic agent is added to enhance color and taste. Sucrose and or any other sweeteners approved by SSMO are allowed to be added to the drink.

2.1.2 History of carbonated soft drinks

Naturally occurring carbonated mineral waters have been known for a long time. These effervescent waters exist as a consequence of excess carbon dioxide in an aquifer dissolving under pressure. Although claims for the medicinal properties of these mineral waters have been grossly exaggerated, the presence of carbon dioxide does make aerated waters and soft drinks both more palatable and visually attractive: the final product sparkles and foams. The first noncarbonated soft drinks appeared during the seventeenth century. In 1767, Joseph Priestley produced the first man-made, palatable carbonated water. Three years later a Swedish chemist, Torbern Bergman, invented a process that produced carbonated water from the reaction between chalk and sulphuric acid, allowing the commercial production of aerated mineral water. In 1783, Jacob Schweppes, a young watchmaker and amateur scientist, perfected an efficient system for manufacturing carbonated mineral water and founded the Schweppes Company in Geneva. He relocated to Drury Lane, London, England in 1790. Since then, the addition of flavorings to aerated waters has seen the development of major soft drinks brands throughout the world. To meet the need for carbonated soft drinks, the soda fountain was developed by Samuel Fahnestock in the United States in 1819. The patenting of the Crown cork by William Painter in 1892 and the automatic production of glass bottles using a glass-blowing machine by Michael J. Owens in 1899 were notable achievements that at last allowed carbonated soft drinks to be

successfully bottled without significant loss of carbonation. Since then, developments in closure technology, polyethylene terephthalate (PET) bottle production, can design and manufacture, syrup making methods, carbonation technology and filling machine manufacture have led to the worldwide beverage industry as we know it today (Ashurst, 2005).

Twenty years ago this was not always the case – carbonated drinks were often produced in old buildings where cross-contamination could easily occur. Filling technology has progressed rapidly to meet the needs of manufacturers and consumers alike. Whilst the basic counter pressure filler is still the main work horse, new generations of electronically and pneumatically controlled filling machines have been developed that allow production under much more hygienic conditions and to much higher standards of filling accuracy and repeatability (Steen and Ashurst, 2006).

2.1.3 Components of carbonated soft drinks

Present-day standards in most countries demand that food and drink, in general, must be of a defined quality and present no health risk to the consumer. It is therefore essential that at every stage of production correct procedures are adopted and ingredients are selected to meet legal requirements of purity and to conform to the legislative controls that apply to them.

Table 1: Soft drink components, general usage and contribution

Component	Typical use level
Water (quality must meet rigid requirements)	Up to 98% v/v when high-intensity sweeteners in use
Bland carrier for other ingredients. Provides essential hydration effects to enable body metabolism.	
Sugars	7–12 % m/v when sole source of sweetener
Contribute sweetness, body to drink. Act as synergist and give balance to flavour	
Fruit juice	Widely variable usage. Usually up to 10% as natural strength, although some specialised lines in this
Provides fruit source identity, flavour, mouthfeel effects. Also contributes to sweetness and acidity.	
High-intensity sweeteners	Use based upon sucrose equivalence (e.g. aspartame might be employed at 0.40–6% m/v as sole sweetener)
Provide sweetness, calorific reduction. Synergist action. Often used in combination e.g. aspartame with acesulfame K	
Carbon dioxide	0.30–6% m/v
Provides mouthfeel and sparkle to drink (carbonates only)	
Acids (e.g. citric)	0.05–0.03% m/v
Contributes sharpness, sourness, background to flavour. Increases thirst-quenching effects	
Flavours	Nature-identical and artificial: 0.10–28% m/m Natural: up to 0.5% m/m
Provide flavour, character and identity to the drink	
Emulsion (flavour, colour, cloud etc.)	0.1% m/v
Carrier for oil-based flavours or colours. Gives cloudy effect in drink to replace or enhance cloud from natural juices	
Colours (natural or synthetic)	0–70 ppm
Standardise and identify colour tone of drink	
Preservatives	Statutory limits apply (e.g. sorbic acid up to 250 ppm in EU)
Restrict microbial attack and prevent destabilisation of the drink	
Antioxidants (e.g. BHA, ascorbic acid)	Less than 100 ppm, subject to user-country legislation
Prevent oxidation, limit flavour and colour deterioration	
Quillaia extract (saponins)	Up to 200 mg/l (EU), up to 95 mg/l (USA)
Acts to provide heading foam, mainly of use in carbonates	
Hydrocolloids (mucilaginous gums)	0.1–0.2% per GMP, minimum amount required to create desired effect
Carrageenans, alginates, polysaccharides, carboxy methyl cellulose etc. Provide mouthfeel, shelf-life stability, viscosity	
Vitamins/Minerals	ADI applies
Used in 'healthy-living' drinks to provide nutritional requirements	

- Sourse: Steen and Ashurst (2006).

Carbonated soft drinks consist of:

Water:

Water, as the main component of a soft drink, usually accounts for between 85 and 95% of the product and acts as a carrier for the other ingredients. Its quality must conform to rigid requirements and not interfere with the taste, appearance, carbonation or other properties of the drink. Subject to the location of the bottling plant, the source of water and product specifications, it may be necessary to carry out treatment to improve the quality of the water used in the manufacture of soft drinks.

In most urbanized areas of the world, public water supplies can meet consumer requirements of potability, but for the soft drinks manufacturer this is not always a suitable qualification for use of the water as a raw material. Most soft drinks factories will carry out their own treatments to counteract the likelihood of a possible change in quality. This is most important in areas where variations are introduced as a result of the use of a national grid system for water supply.

In less developed countries, water treatment becomes an essential prerequisite where microbial loading could provide cause for concern. It is necessary for a full water treatment to be effective and to ensure the wholesomeness of water supplies for boiling purposes (Ashurst, 2005).

Within Europe the minimum requirement is that all water used in a soft drinks factory must comply with the European commission Drinking Water Directive 80/778/EEC. All major producers of carbonated soft drinks have their own water standard. This usually requires treatment of all incoming water, except in the case of natural mineral water, where no treatment is allowed. If the water supply is from the local water company, it is imperative that a good working relationship is set up. At certain times, owing either to maintenance or to drought, the source of the water supplied can change. Even

though water companies have a statutory obligation to ensure that the water they supply is fit for use, this is to a much lower standard than is required to produce a soft drink, which must taste the same wherever and whenever it is made (Steen and Ashurst, 2006).

Water Requirements:

Water should comply with the following quality requirements. It should be free from:

- High levels of elements and mineral salts.
- Objectionable tastes and odors.
- Organic material.

It should also be

- Clear and colourless.
- Free from dissolved oxygen.
- Sterile, that is, free from microorganisms.

Ideally, a non-variable supply of water should be available at all seasons of the year to allow a standard manufacturing process to be established (Ashurst, 2005).

Carbon dioxide:

It had been recognized by many scientists in the early 1700s that the gas produced by brewery fermentation, combustion of wood and addition of acids to chalk/marble was one and the same. It was given several names including artificial air, mephitic air, fixedair, gas acide carbonique (and finally gaz oxide de (Steen and Ashurst, 2006).

Table 2: limits of carbon dioxide:

Drink	Gas volume
Natural carbonated soft drinks	Must not be less than two times the beverage inside the bottle under normal atmospheric pressure.
Artificial carbonated soft drinks	Must not be less than two times the beverage inside the bottle under normal atmospheric pressure.
Soda water	Three parts gas to one part beverage inside bottle.

- Source: SSMO (2007).

Carbon dioxide levels vary widely and are usually expressed as 'volumes of CO_2 gas' (i.e. the volume of carbon dioxide contained in solution in one volume of product). Lightly carbonated products will contain around 2.0–3.0 volumes of the gas; moderate carbonation usually refers to about 3.5–4.0 volumes and high carbonation levels are around 4.5–5.0 volumes. Large bottles that are likely to become part full will be relatively highly carbonated, and mixer drinks contain among the highest carbonation levels because the resultant mixture (e.g. gin and tonic) needs to have a satisfactory residual level of dissolved carbon dioxide (Ashurst, 2005).

Carbonation is the impregnation of a liquid with carbon dioxide gas. Carbon dioxide is a non-toxic, inert gas that is virtually tasteless and is readily available at a reasonable cost. It is soluble in liquids (the degree of solubility increasing as the liquid temperature decreases) and can exist as a gas, liquid or solid. When dissolved in water it forms carbonic acid. It is carbonic acid in combination with the product that produces the acidic and biting taste found in carbonated waters and soft drinks. Above a certain level of carbonation, carbon dioxide also has a preserving property, which is a bonus from its use. Carbon dioxide gas is heavier than air; it has a specific gravity of 1.53 under normal conditions of temperature and pressure. It has a molecular weight of 44.01 and does not burn, although it will support the combustion of magnesium (Ashurst, 2005).

It is a fairly stable compound that decomposes into carbon and oxygen only at very high temperatures (Ashurst, 2005).

Sweeteners (sweetening agents)

The use of carbohydrate sweeteners in juices and drinks has increased ever since the times of Captain Cook, when sugar was used to preserve juices. Sugar (sucrose) is still regarded as the 'gold' standard for taste delivery and mouthfeel. Carbohydrate-based sweeteners still represent the largest share of the global sweetener market and currently account for 81% of sweetener usage (Cosgrove, 2003).

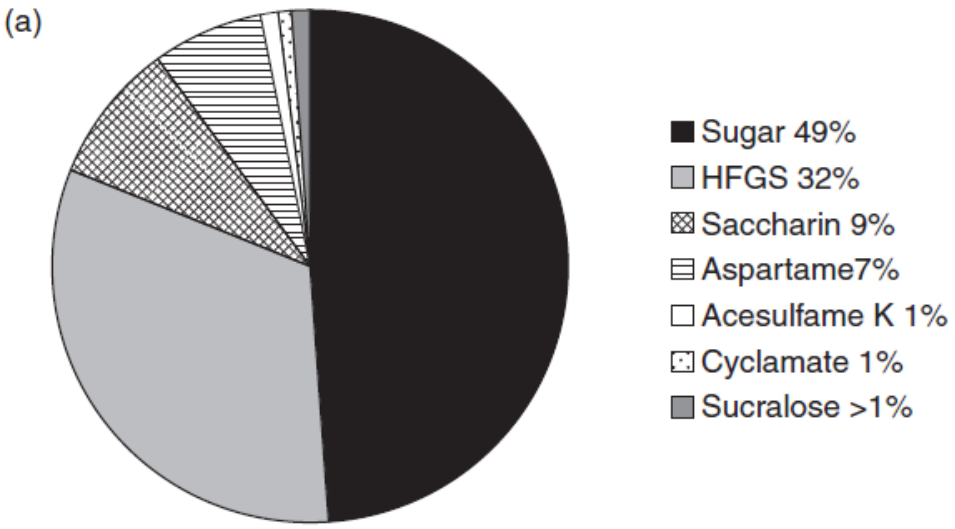

Figure 1: Estimated global sweetener market

- Source: (Cosgrove, 2003).

One of the major drivers of growth in carbonates has been the development of sweeteners and consequent improvement in the quality of low calorie soft drinks, particularly in the USA and UK. Saccharin was invented in about 1874 and very rapidly became popular as a sweetener for soft drinks, usually blended into sugar to reduce cost (Steen and Ashurst, 2006). Sucrose is regarded as the 'gold' standard for a sweet taste. It is manufactured from cane or beet and available in crystalline or liquid form.

Sucrose is a disaccharide with a molecular weight of 342.31. It is available in a very pure state and in a variety of physical forms (Ashurst, 2005).

Intense sweeteners:

The use of intense sweeteners in soft drinks has increased dramatically over the period 1985–2004. Saccharin was the first high-intensity sweetener to be marketed and its usage increased during the First World War as a result of sugar scarcity. Cyclamate entered the UK market during the 1960s, but was controversially banned in many countries as it was thought to be a potential carcinogen.

The 1970 cyclamate ban ended the use of saccharin/cyclamate blends in many soft drinks markets. The effect of this was that the low-calorie soft drinks market remained small and static owing to the poor taste quality of products available. The introduction in 1982–83 of aspartame in particular, and acesulfame K to a lesser extent, into the global soft drinks market dramatically improved the taste quality of sugar-free soft drinks formulations. There followed a period of rapid growth in the low-calorie sector. The use of intense sweeteners in soft drinks was given a further boost in the UK market when, in 1995, the requirement for a minimum carbohydrate level of 4.5_Brix in non-low-calorie products was removed. Products were reformulated to incorporate blends of intense sweeteners and low levels of carbohydrate sweeteners (around 0.5–3.0_Brix) to deliver cost savings without compromising taste quality. Over time, and as the use of intense sweeteners expanded, optimization of the sweetener blends continued to deliver excellent tasting products. Currently, in the UK market, 50% of all beverages contain intense sweeteners, even though the diet market is only 25% of the total (Cosgrove, 2003). In the United Kingdom, Diet Coke now outsells regular Coke (Grocer, 2003).

Acidulants (Acid agents)

The use of acidulants is an essential part of beverage formulation, with the acid component usually third in order of concentration. Acidulants performs a variety of functions in addition to their primary thirst quenching properties, which are the result of stimulation of the flow of saliva in the mouth. Because it reduces pH, an acidulant can act as a mild preservative and in some respects as a flavour enhancer, depending on the other components present.

In carbonated beverages there is the additional effect of dissolved carbon dioxide gas. Although it is not officially recognized as an acidulant, the inclusion of carbon dioxide, under pressure, will certainly provide extra sparkle, mouthfeel, flavor and sharpness in a drink (Ashurst, 2005).

Table 3: the most encountered acidulatnts in the beverage industry

Acidulant	Molecular weight	Melting point (°C)
Citric acid, 2-hydroxy-1,2,3-propane tricarboxylic acid, $HOOCCH_2C(OH)(COOH)CH_2COOH$	192.1	152–154
Tartaric acid (D-tartaric) 2,3-dihydroxy butanedioic acid HOOCCH(OH)CH(OH)COOH	150.1	171–174
Phosphoric acid orthophosphoric acid H_3PO_4	98.0	42.35
Lactic acid (DL-lactic) 2-hydroxy propanoic acid $CH_3CH(OH)COOH$	90.1	18
Malic acid (D-malic) 2-hydroxy butandioic acid $HOOCCH(OH)CH_2COOH$	134.1	98–102
Fumaric acid *trans*-butenedioic acid HOOCCH=CHCOOH	116.1	299–300
Acetic acid ethanoic acid CH_3COOH	60.0	16–18

- Source: Ashurst (2005).

Flavours and colours:

It is the flavor of a drink that provides not only a generic identity but also its unique character. This part of the sensory profile is responsible for pleasing and attracting the consumer. For example, having decided on a Cola drink, the consumer will be able to differentiate between colas by virtue of the background flavouring components, which collectively provide a reference point to which the consumer can return, consciously or not, on future occasions whenever a particular brand of drink is selected. A flavouring consists of a mixture of aromatic substances carefully balanced to convey the right message to the sensory receptors of the consumer. The preparation of such a mixture is a serious matter; the soft drinks flavourist, like the perfumer, must be well versed in the technique, be creative and be able to translate ideas into a practical solution (Ashurst, 2005).

As previously mentioned the original carbonates were artificial imitations of naturally occurring mineral waters. Manufacturers blended mineral salts in the same proportions as found in the natural spring waters and added carbonated water. A large range of such waters was available during the early 1800s. Early attempts at producing flavoured products were limited by a lack of stable flavourings and spoilage problems. The flavoring materials used consisted mainly of herbal/botanical extracts, for example, ginger, nettle, nutmeg, horehound, lemon oil, vanilla etc., but the technology for manufacture of soluble stable flavouring extracts developed rapidly during the middle of the century with the establishment around this time of many specialty flavour companies such as W.J. Bush and Stevenson & Howell in London (Steen and Ashurst, 2006).

Flavourings for soft drinks are of two main types: water-miscible and water-dispersible. Water-miscible flavourings are formulated to dissolve easily in water, forming a clear bright solution at dosages usually in the region of 0.1%. They typically contain mainly oxygenated, highly polar

compounds. Water-dispersible flavourings are strictly speaking ‘insoluble’, having in their makeup a relatively non-polar oil phase, usually citrus, which conveys the characteristic zest-like contribution from the peel. This type of flavour is introduced in the form of an emulsion, enabling oil-based flavouring substances to be incorporated in a soluble form (Ashurst, 2005).

Colours:

Those of us fortunate enough to possess optical powers capable of distinguishing a variety of colours will appreciate the influence that this particular sensory dimension exercises on our judgment of matters important to our well-being, such as food and drink. The perception of colour influences a taster’s reception of a drink, and as a consequence there is inevitably some controversy over the use of colourings in food and drink (Ashurst, 2005).

Colour provides a means of presenting a beverage to the consumer so that the perceived organoleptic attributes are correctly placed in an ordered sequence of appreciation. Both quality and quantity of colour are of importance, and certain colours will provoke, or perhaps complement, a particular taste. Reds will favor the fruitiness of soft drinks, for example, blackcurrant, raspberry and strawberry. Orange and yellow tend towards the citrus flavours. Greens and blues reflect the character of peppermints, spearmint and cool flavors, sometimes herb-like and balsamic flavours. Browns align with the heavier flavours, for example, colas, shandies, dandelion and burdock. Therefore, the deceit, if ever intended, is aimed at ensuring that consumers are able to maximize their enjoyment of the beverage (Ashurst, 2005).

Table 4: Artificial (synthetic) colours permitted in soft drinks.

Colour	E-no.	Colour stability			Colour contribution
		Light	Heat	Acids	
Quinoline yellow	E104	Good	Good	V. good	Greenish yellow
Tartrazine (FD&C yellow no. 5)	E102	Good	Good	V. good	Lemon yellow
Sunset Yellow (FD&C yellow no. 6)	E110	Good	Good	V. good	Orange shade (similar to orange peel)
Carmoisine (azorubine)	E122	Good	Good	Good	Bluish red
Ponceau 4R	E124	Good	Good	Good	Bright red
Patent blue FCF	E131	Good	Good	Poor	Bright blue
Indigotine (FD&C blue no. 2)	E132	Fair	Poor	Fair	Dark bluish red
Brilliant blue FCF (FD&C blue no. 1)	E133	Good	Good	Good	Greenish blue
Green S	E142	Fair	Good	Good	Greenish blue

- Source: Ashurst (2005).

Food colours are broadly divided into two classes: natural and artificial. In the United States, these are listed as either 'exempt from certification' or 'certified'. The natural colours are botanical extracts, with the exception of carmine (a red color), which should perhaps be termed an entomological extract as it is obtained from the insect *Dactilopius coccus*, sometimes termed the cochineal beetle, which breeds and feeds on particular cacti indigenous to Central and South America. Table 5.6 lists artificial colors permitted in soft drinks under European union legislation. (Ashurst, 2005).

Preservatives:

A preservative can be defined as any substance that is capable of inhibiting, retarding or arresting the growth of micro-organisms or any deterioration of food due to micro-organisms or as masking the evidence of any such deterioration. In the European Union, defined maximum levels of permitted preservatives are given according to the food substrate concerned. For soft drinks that can be consumed without dilution.

Table 5: the most common preservatives and their salts.

Preservative	E-no.	Alternative form used at equivalent level	E-no.
Benzoic acid (m.p. 122°C) C_6H_5COOH *Benzene carboxylic acid*	E210	Sodium benzoate Potassium benzoate Calcium benzoate	E211 E212 E213
Sorbic acid (m.p. 133°C) $CH_3CH=CH_2–CH_2=CHCOOH$ *2,4-Hexadienoic acid*	E200	Sodium sorbate Potassium sorbate Calcium sorbate	E201 E202 E203
Sulphur dioxide (gas) SO_2 *Sulphurous anhydride*	E220	Sodium sulphite Sodium hydrogen sulphite, sodium bisulphite Sodium metabisulphite Potassium metabisulphite Calcium sulphite Calcium hydrogen sulphite, calcium bisulphite Potassium bisulphite	E221 E222 E223 E224 E226 E227 E228

- Source: Ashurst (2005).

Benzoic acid and benzoates:

Benzoic acid occurs naturally in some fruits and vegetables, notably in cranberries, where it occurs in amounts of the order of 0.08% m/m. It is also found in some resins, chiefly in gum benzoin (from *Styrax benzoia*), and in coal tar. Commercially available benzoic acid is produced by chemical synthesis. Benzoic acid is generally considered to exhibit an inhibitory effect on microbial growth, although it is of little use for bacterial control, where the greatest problem will occur at pH values above 4, outside the effective limit mentioned above. Improved results are obtained when it is used in conjunction with other preservatives, for example, SO_2 or sorbic acid, due to synergistic effects. It is interesting to note that the current European Directive, which sets individual limits of 300 mg/l for sorbic acid and 150 mg/l for benzoic acid in non-alcoholic flavoured drinks, nevertheless permits a joint preservative use of up to 250 mg/l sorbic acid with 150 mg/l benzoic acid (Ashurst, 2005).

Sorbic acid and sorbates

Sorbic acid is found naturally in a number of fruits and vegetables, notably in the juice of unripe mountain ash berries (from *Sorbus aucuparia*), where it occurs together with malic acid. Sorbic acid and its salts are among the most widely used preservatives in the world. In soft drinks the most commonly used form is potassium sorbate because, like benzoic acid, there are problems in preparing its solution (the solubility of sorbic acid _ 0.16% m/v at 20_C). In common with benzoic acid, as a microbial inhibitor, sorbic acid and its sorbates show reduced effectiveness with increased pH. Although activity is greatest at low pH values, sorbates have the advantage of being effective at pH values as high as 6.0–6.5, in contrast with benzoic acid, for which the comparative range is pH 4.0–4.5. The undissociated form, as with benzoic acid, is primarily responsible for its preservative action Ashurst, 2005).

2.1.4 Production of carbonated soft drinks

Ashurst (2005) has described in his book -the chemistry and the technology of soft drinks and juice- the process for making a carbonated soft drink as follows:

Syrup preparation

Most products are traditionally prepared as a syrup-plus-water mix, in a ratio of some 1 part (volume) syrup to between 3 and 6 parts (volume) water. This allows a concentrated batch of syrup to be made and then proportioned with water to form the final product. For a sugar-based product the syrup would typically consist of 67_Brix sugar, citric acid, flavourings, colourings, preservatives and water. The ingredients are carefully weighed out and added to the mixing vessel. The syrup is pre-prepared and fully tested before being sent to the proportioner for mixing with water and subsequent carbonation.

This is carried out in the syrup room as a batch process, allowing the multitude of soft drink flavours to be catered for (Ashurst, 2005).

De-aeration

Why de-aerate? As discussed earlier, the presence of air in a product causes product deterioration, as well as giving a false reading of the level of carbon dioxide present due to the partial pressures involved. Experience has shown that the aim should be to reduce the level of air within a product to below 0.5 ppm wherever possible. In this way the product will be at minimum risk from deterioration due to the presence of oxygen; hence shelf life will be improved and filling problems minimized. The presence of air and carbon dioxide causes nucleation sites within the products, giving rise to the phenomenon known as fobbing. The higher the air content the more difficult it is to hold carbon dioxide in solution (Ashurst, 2005).

Carbonators

The final product is fed to a vessel pressurized with carbon dioxide gas. The rate of flow and the pressure of the carbon dioxide are critical to ensure the correct carbonation level. The greater the liquid surface area exposed to the carbon dioxide, the higher the rate of absorption of the carbon dioxide by the liquid. The carbon dioxide is often sparged into the liquid under pressure; this allows small bubbles of gas to be formed which can be easily absorbed by the liquid. The higher the pressure, the smaller the gas bubbles formed at the sparger and the greater the gas bubbles' surface area made available for the gas to be absorbed by the liquid (Ashurst, 2005).

Filling principles

A carbonated product made to specification has then to be filled into the required container at a commercially viable filling rate. This is achieved under gravity, the rate of flow being dependent on the head difference

between the filler bowl and the container. The rate of flow will increase if an overpressure is introduced. The pressure from the top of the filling bowl to the outlet of the filling valve provides the driving force to fill the container.

In actual practice, the rate of flow would probably be turbulent and proportional to rather than to the pressure alone. It does demonstrate that for viscous liquids it is necessary either to increase the filling tube diameter or to increase the driving pressure to maximize the flow rate through an orifice. Considering the process in more detail reveals some of the problems facing the filler designer, especially with regard to how the process is controlled. It is simple to envisage how a container is filled under gravity alone: it is the same as filling a bottle from the kitchen tap. To control the process under pressure with carbonated product is more complex. However, if the pressure in the container and the pressure of the gas in the filler bowl headspace are the same, gravity filling conditions will apply. This is exactly what is done (Ashurst, 2005).

2.1.5 Nutritional value of carbonated soft drinks

There are three main areas of particular nutritional significance for soft drinks. The first is energy. Some soft drinks are formulated to deliver a rapidly assimilated energy boost to the consumer. All carbohydrates are important sources of energy but soft drinks generally contain soluble sugars, which are easy to administer. However, because high levels of sugars are often intensely sweet and even sickly, with a cloying sensation in the mouth, energy drinks are formulated around glucose syrup. For a given solid carbohydrate content, this raw material is much less sweet than sucrose. Selection of the method of hydrolysis used for the corn starch allows glucose syrup to be tailored, to some extent, to include mixed carbohydrates, that is, mono-, di-, tri-and oligosaccharides. Such blends are the basis of some very effective products used by athletes and those recovering from illness (Ashurst, 2005).

The second area of nutritional significance is that of the so-called isotonic drinks, which are of equivalent osmolality to body fluids. They promote extremely rapid uptake of body salts and water, and are very important products for sportspeople and others requiring almost instant hydration. Third, soft drinks have been widely formulated to low-calorie forms and these are now available for those who wish to enjoy such beverages and yet minimize their calorific intake.

Other nutritional benefits that are claimed by some producers include the delivery of essential vitamins and minerals, especially to children. On the negative side, soft drinks have acquired a reputation for being an agent in the development of dental caries. This has been claimed to arise when sugar residues remain in the mouth or when (especially) young children have an acidic drink almost constantly in their mouths. It is perhaps now accepted that the dental caries problem is related more to the misuse, or even abuse, of soft drinks than to the effects of normal consumption of such products (Ashurst, 2005).

The nutritional value of soft drinks is sometimes exaggerated by manufacturers who want consumers to perceive their products to be of special benefit. That said, the value of soft drinks must not be understated, because they are an essential vehicle for hydration. Soft drinks are usually absorbed more readily than water (because of their osmolality), can replace lost salts and energy quickly and are rapidly thirst quenching. Their balance of sweetness and acidity, coupled with pleasant flavors, makes them attractive to all ages of consumers. Products are specially formulated to meet the tastes, nutritional needs and physiological constraints of the whole population, from babies to geriatrics. The claims that are legally permitted for soft drinks vary from country to country but for the most part are limited to nutritional claims concerning energy, proteins, vitamins and/or minerals. Any form of medicinal claim (i.e. curative or symptomatic relief) will almost

always be excluded by corresponding medicines legislation. There is, nevertheless, a growing trend to include natural extracts in many soft drinks (e.g. ginseng or ginkgo) and then rely on the general understanding and folklore that surrounds such ingredients to impart the special values that have been attributed to them (Ashurst, 2005).

2.1.6 Microbiology of carbonated soft drinks

According to Sudanese standards, (SSMO, 2007) a carbonated non-alcoholic soft drink must not contain *E.coli* bacteria, pathogenic bacteria and yeast and mold cells.

The Sudanese standard also states that carbonated non-alcoholic drinks must be bottled under hygienic conditions including; buildings, equipment, packing and packaging materials and machinery used.

Although there is little evidence of the formation of toxic fermentation products in beverages, the problem of spoilage frequently arises .Because of their utilization of sugars; yeasts are of most immediate concern. Yeasts are classified with the fungi and are unicellular for most of their life cycle. Together with molds and bacteria they can bring about deterioration in flavour, producing taints, off-notes and differences in mouth feel. Most yeast can grow with or without oxygen, whereas most bacteria cannot survive in oxygen. The majority of yeasts thrive at temperatures between 25 and 27◦C; some can survive at temperatures over 70◦C and others can exist, apparently quite comfortably, at 0–10◦C. Bacteria exhibit certain deviations in characteristics, with an optimum growth temperature at around 37◦C. Soft drinks provide an ideal growth substrate for many micro-organisms, given adequate supplies of the required nutrients. Apart from water, the environmental necessity, typical requirements are sources of carbon (carbohydrates), nitrogen (amino acids), phosphorus (phosphates), potassium, calcium (mineral salts) and traces of other minerals, for example, sulphur,

iron, cobalt and even vitamins. Because of the obvious link with protein formation during cell growth, the presence of combined nitrogen is of particular importance. Also, where they are introduced to beverages via fruit pulp or caramel (colouring), there will be a great susceptibility to spoilage by certain micro-organisms. Perhaps the most difficult aspect of dealing with microbial contamination in soft drinks relates to the delay factor when an apparently good quality product leaves the bottling line for storage and distribution only to be returned later, maybe after several weeks, when severe deterioration in performance has taken place. Fortunately, such occurrences are rare in the modern soft drinks industry, but to any manufacturer this is a nightmare scenario that must be avoided at all costs (Steen and Ashurst, 2006).

Drink constitutes a unique system, which can inhibit or enhance the growth of micro-organisms. Micro-flora, if present, will enter a dormant stage during which their chances of survival are assessed in relation to the immediate surroundings. Following this 'lag' stage, during which specific micro-flora may adapt to their new environment and start to grow, there is a burst of species-dependent activity, during which the population doubles repeatedly at a steady rate. As a bottled drink is a 'closed' system, waste products and diminishing nutrients will serve to slow down the growth and eventually bring it to a standstill, at which point the death rate increases and all activity stops. Although the product is perhaps not a health hazard, it has been spoiled and can no longer satisfy its intended function (Steen and Ashurst, 2006).

Carbon dioxide, though not added specifically as a preservative, contributes to the inhibition of the growth of micro-organisms and, coupled with other factors (e.g. pH), contributes to the stability of a drink. Carbon dioxide is deemed to be effective at volumes over 2.5 or 3.0, and for this reason the incidence of spoilage in carbonated beverages is less than that in

noncarbonated versions. ('Volumes' of CO_2 in general terms refers to the number of times the total volume of the gas, adjusted to 760 mmHg and 0ºC, can be divided by the volume of liquid in which it is dissolved) (Ashurst, 2005).

Although preservatives can be used to good effect in beverage formulations they should never be considered infallible, and there is no substitute for stringent quality and hygiene controls at every stage of manufacture. Within their own product specification, raw materials should be assigned workable limits for microbial activity so that there is little chance of excessive contamination in the finished beverage product. Equally, all processing plant, machinery and containers likely to come into contact with the product during manufacture should undergo a thorough cleaning (sanitization) before use. Certain strains of yeast, molds and bacteria can survive in relatively low pH conditions and some of these can exist and grow in the presence of certain preservatives; so it is important that everything is done to prevent their multiplying. Under favorable conditions, a typical rapidly growing yeast strain can double its numbers every 30 min, and at this rate in 12 h one yeast could become 16.7 _ 106 cells, provided no inhibitory factor is present (Ashurst, 2005).

Good hygienic practices and adherence to GMPs are the most effective control measures for microbial contamination in the soft drinks industry, particularly for yeasts. Sticky sugar and fruit residues are ideal food sources for yeasts and molds. The Hazard Analysis and Critical Control Point (HACCP) approach has been adopted by food processors around the world. In the United States, HACCP is mandatory for fruit juice processors, with good agricultural practices (GAP) as the foundation of a successful HACCP system. In Europe, growers, distributors and packaging houses must meet the EUREGAP protocols if they wish to be certified to sell their products to certain markets or established buyers (Stier and Nagle, 2003).

2.1.7 Quality control of carbonated soft drink

It is a fundamental requirement of any food process that the food produced should be safe for consumption. Food safety is a basic need but there is a danger that it may be overlooked in the development of effective and efficient processes. There are three key elements to ensuring food safety is achieved in food manufacture:

1. Safe design of the process, recipe and packaging format;
2. Prerequisite programs or good manufacturing practice to control the manufacturing environment;
3. Use of the HACCP system of food safety management (Brennan and Grandison, 2011).

Although the primary function of food for humans is survival, it now has the additional associations with health, enjoyment and acceptability. Today's consumer looks to suppliers and manufacturers for a product with which there is no associated risk in consumption and which is marketed in accordance with strict observance of the laws governing food safety (Brennan and Grandison, 2011).

However, to be assured of complete safety, it is necessary to look further, into the actual ingredient makeup of the drink itself. The safety of food additives and other ingredients is monitored according to guidelines issued by the joint committee of JECFA, WHO and the (Brennan and Grandison, 2011).

Knowledge of health safety is gained primarily as a result of animal feeding trials coupled with relevant short- or long-term toxicological investigations. In later stages of testing, humans may also be included in the studies to ascertain that their physiological reactions are similar to those found in animals and level of intolerance to food additives of 0.026% was given in the report, which is equivalent to about 3 people in 10,000 of the population being affected (Ashurst, 2005).

2.1.8 General requirements of the Sudanese standard SSMO (250/2007):

SSMO (2007) states the general requirement of carbonated soft drinks in Sudan as follows:

Water used in preparing non-alcoholic carbonated soft drinks must comply with the Sudanese standard. Sugar and any other sweeteners must be certified by the Sudanese standard and metrology organization and surly must be within the limit assigned for any of them it's accepted to use colouoring, flavouring and aroma agents that is proven not to damage health and which are generally accepted to be used in foodstuff.

Natural fruit oil can be used in preparing carbonated soft drinks.

Synthetic agents are also allowed to be used in condition of being verified by the organization. Calcium carbonate can also be used in the preparation of soda water but shall not exceed 1 gram per liter calculated as calcium carbonate. Non-Alcoholic soft drinks shall be free from precipitants, fermented substances, rotten substances, sabonin, saccharin, cyclamate and mineral acids "except of phosphoric and sulfuric". Non-Alcoholic soft drinks shall be free from any heath deteriorating substances "chemical or biological". And shall be free from pathogenic microbes.

According to Sudanese standards, (SSMO,2007) carbonated non-alcoholic soft drinks shall be packaged in glass or cans made of raw materials certified by SSMO. And it must be tightly closed isolated from air and microorganisms.

Packaging requirements

The following shall be declared:

- Product name and trade mark.
- Name of producer/manufacturer and address.
- Minimum filling volume in container.

- Detailed components description and concentration.
- Production date.
- In non-glass containers; production and expiry dates must be declared.

2.2 Prerequisites' of ISO 22000:2005

2.2.1 Good mmanufacturing practice (GMP)

2.2.1.1 Definition

Prerequisite programs or 'Good Manufacturing Practice' (GMP) provide the hygienic foundations for any food operation.

The terms 'prerequisite programs' and 'Good Manufacturing Practice' are used interchangeably in different parts of the world but have the same general meaning. For simplicity, the term prerequisite programs will be used. (Brennan and Grandison, 2011).

Good Manufacturing Practices (GMPs) as defined by the Food and Drug Administration in 21 CFR part 110 are the minimum sanitary and processing requirements for food companies. GMPs are fairly broad and general and can be used to help guide the development of Standard Operating Procedures (SOPs) which are very specific (FDA, 1986).

Brennan and Grandison (2011) also adds that, several groups have suggested definitions for the term prerequisites and the most commonly used are reproduced here.

Prerequisite programs are:

- Practices and conditions needed prior to and during the implementation of HACCP and which are essential to food safety (WHO, 1999).
- universal steps or procedures that control the operating conditions within a food establishment, allowing for environmental conditions that are favorable for the production of safe food (CFIA, 2000)

Procedures, including GMP, that address operational conditions, providing the foundation for the HACCP system (NACMCF, 1997).

Good hygienic practices and adherence to GMPs are the most effective control measures for microbial contamination in the soft drinks industry, particularly for yeasts. Sticky sugar and fruit residues are ideal food sources for yeasts and molds (Stier and Nagle, 2003).

GMPs are programs that comprise the basic, universal steps and procedures that control operating conditions within establishments and ensure favorable conditions for the production of safe food. HACCP systems relate to hazards within a specific process. GMPs are the control factors that relate to the entire operation and are not process-specific. GMPs include such programs as pest control, recall procedures, construction/maintenance and sanitation. (AMIF, 1997).

The AMIF also adds that In order to ensure that GMPs are carried out, there are step-by-step descriptions that instruct individuals as to how, when and what tasks are to be performed for a required GMP. The FSIS Pathogen Reduction/HACCP rule published in July, 1996 combines the concepts of Hazard Analysis and Critical Control Point systems with the FSIS requirement for written Sanitation Standard Operating Procedures (SSOPs). In order to avoid confusion, it is important to have a clear understanding of how the HACCP concepts relate to SSOPs as well as the relationship between the SSOPs, Good Manufacturing Practices (GMPs), and HACCP plans that many companies already have in place.

Before developing a HACCP plan, it is essential to have a sound base of good hygienic and manufacturing practice. This means that all basic hygienic practices, encompassing facilities and operations, need to be in place and operating effectively. These practices include programs In relation

to the implementation of HACCP the documented procedures are referred to as prerequisite programs (MAF, 1997).

MAF proceeds, Prerequisite programs cover all those activities which interact within and across various processes that may influence the food safety outcomes of the product. Confirmation that effective prerequisite programs are in place means that the HACCP team can focus on the application of HACCP to the product and process selected, without repeating the analysis of hazards from the processing and surrounding environment. The prerequisite programs may be generic to all processes at an individual premise.

2.2.1.2 Establishment of (GMP)

Prerequisite programs should include the following:

- Cleaning and sanitation (hygiene of facilities and equipment, including both preoperational and operational).
- Hygiene of personnel (training, health, personal habits and protective clothing).
- Reception of raw material.
- Incoming materials (ingredients, food additives, wrapping and packaging).
- Product recall.
- Repairs and maintenance.
- Storage and transport, including temperature controls.
- Training.
- Vermin control.
- Waste management.

Establish prerequisite programs *(PRPs)* Basic prerequisite programs should be in place to:

- Protect products from contamination by biological, chemical and physical food safety hazards
- Control bacterial growth that can result from temperature abuse and maintain equipment.

Table 6: Prerequisite program topics for manufacturing facilities and recommended general principles of food hygiene for primary production facilities).

Establishment: design and facilities	**Control of operation**
Location	Control of food hazards
Premises and rooms	Key aspects of hygiene control systems
Equipment	Incoming material requirements
Facilities	Packaging
	Water
	Management and supervision
	Documentation and records
	Recall procedures
Establishment: maintenance and sanitation	**Establishment: personal hygiene**
Maintenance and cleaning	Health status
Cleaning programmes	Illness and injuries
Pest control systems	Personal cleanliness
Waste management	Personal behaviour
Monitoring effectiveness	Visitors
Transportation	**Product information and consumer awareness**
General	Lot identification
Requirements	Product information
Use and maintenance	Labelling
	Consumer education
Training	
Awareness and responsibilities	
Training programmes	
Instruction and supervision	
Refresher training	

- Source: Brennan and Grandison (2011).

Detailed explanation of above table as follows:

Control of operation the rationale for operational control listed in table is "to reduce the risk of unsafe food by taking preventive measures to assure the safety and suitability of food at an appropriate stage in the operation by controlling food hazards". This includes the need to control potential food hazards by sing a system such as HACCP.

Key aspects of hygiene control systems, including:

- time and temperature control
- Microbiological and other specifications;
- microbiological cross-contamination risks;
- Physical and chemical contamination.

Incoming material requirements and systems to ensure the safety of materials. Ingredients at the start of processing are necessary, along with a suitable packaging design. (Codex, 1999).

Codex (1999) lists the importance of hygienic control of water, ice and steam, appropriate management and supervision, the need to keep adequate documentation and records and the need to develop and test suitable recall procedures so that product can be effectively withdrawn and recalled in the event of a food safety problem.

Establishment: maintenance and sanitation

Maintenance and cleaning are important both to keep the processing environment, facilities and equipment in a good state of repair where they function as intended and to prevent cross contamination with food residues and microorganisms that might otherwise build up. Facilities should operate preventative maintenance programs as well as attending to breakdowns and faults without delay. Cleaning programs should be developed to encompass all equipment and facilities as well as general environmental cleaning. Cleaning methods need to be developed that are suitable for the item to be

cleaned, including the use of appropriate chemical cleaning agents, disinfectants, hot/cold water and cleaning tools, e.g. brushes, scrapers, cloths, etc. Methods should describe how the item is to be cleaned and personnel should be trained to apply the methods correctly. A cleaning schedule should also be developed to identify the frequency of cleaning needed in each case and records of cleaning and monitoring should be kept (Codex, 1999).

Cleaning in place (CIP) solutions may be used in certain types of equipment, e.g. tanks and lines. Here it is important that the CIP programs is properly designed for the equipment to be cleaned, taking into account the flow rates, coverage and the need for rinsing and disinfection cycles.

Pest control systems are important to prevent the access of pests that might cause contamination to the product. Pest management is often contracted out to a professional pest control contractor. Buildings need to be made pest proof and regularly inspected for potential ingress points. Interior and exterior areas need to be kept clean and tidy to minimize potential food and harborage sources. Suitable interior traps and monitoring devices should also be considered and any pest infestations need to be dealt with promptly, without adversely affecting food safety (Codex, 1999).

Waste management should ensure that waste materials can be removed and stored safely so that they do not provide a cross-contamination risk or become a food or harborage source for pests.

All maintenance and sanitation systems should be monitored for effectiveness, verified and reviewed, with changes made to reflect operational changes.

Establishment: personal hygiene

The objectives for personal hygiene stated in table are: “To ensure that those who come directly or indirectly into contact with food are not likely to contaminate food by:

- maintaining an appropriate degree of personal cleanliness;
- Behaving and operating in an appropriate manner.”

Food companies should, therefore, have standards and procedures in place to define the requirements for personal hygiene and staff responsibility; and staff should be appropriately trained. This should include the establishment of health status where individuals may be carrying disease that can be transmitted through food, a consideration of illness and injuries were affected staff members may need to be excluded or wear appropriate dressings, the need for good personal cleanliness and effective hand washing, the wearing of adequate protective clothing and the prevention of inappropriate behavior such as smoking, eating or chewing in food handling areas. Visitors to processing and product handling areas should be adequately supervised and required to follow the same standards of personal hygiene as employees.

Transportation To ensure continuation of food safety throughout transportation, transport facilities need to be designed and managed to protect food products from potential contamination and damage and to prevent the growth of pathogens.

This includes the need for cleaning and maintenance of vehicles and containers and the use of temperature control devices where appropriate.

Product information and consumer awareness It is important that sufficient information is easily identifiable on the products so that the lot or batch can be identified for recall purposes and that the product can be handled correctly, e.g. stored at <5_C. Product information and labeling

should be clear such that it facilitates consumer choice and correct storage/use.

Codex (1999) also highlights the importance of consumer education, particularly the importance of following handling instructions and the link between time/ temperature and foodborne illness.

Training Food hygiene training is essential to make personnel aware of their roles and responsibilities for food control. Companies should develop and implement appropriate training programs and should include adequate supervision and monitoring of food hygiene behavior. Training should be evaluated and reviewed with refresher or update training implemented as necessary (Codex, 1999).

2.2.1.3 Validation and verification of prerequisite programs

Prerequisite programs are the basic standards for the food facility, in which the safely designed product can be manufactured. They form the hygiene foundations on which the HACCP System is built to control food safety every day of operation. As such, it is essential that prerequisite programs are working effectively at all times and it is therefore necessary that each prerequisite element is validated to establish that it will be effective and that an ongoing program of monitoring and verification is developed and implemented (Codex, 1999).

2.2.2 Hazard analysis and critical control points (HACCP)

2.2.2.1 Importance of HACCP

The Hazard Analysis and Critical Control Point (HACCP) approach has been adopted by food processors around the world. In the United States, HACCP is mandatory for fruit juice processors, with good agricultural practices (GAP) as the foundation of a successful HACCP system. In Europe, growers, distributors and packaging houses must meet the EUREGAP

protocols if they wish to be certified to sell their products to certain markets or established buyers (Stier and Nagle, 2003).

2.2.2.2 HACCP principles

The application of HACCP is compatible with the implementation of quality management systems such as the ISO 9000 series and is the system of choice in the management of food safety within such systems (Anon, 2000). One of the benefits of the HACCP system is that it focuses attention on areas where problems potentially may occur, and requires that food service facilities be prepared to deal with problems immediately if they occur (Puckett and Schneider, 1997). The HACCP system consists of seven principles. These principles make up the Codex standard, which has become the reference for international food safety and identified as the baseline for consumer protection under the Agreement on Sanitary and Phytosanitary Measures agreed at the General Agreement on Tariffs and Trade (GATT) negotiations in 1995 (Slatter, 2003).

Principle 1 Conduct a hazard analysis:

A hazard analysis is the identification of any hazardous biological, chemical or physical properties in raw materials and processing steps, and an assessment of their likely occurrence and potential to cause food to be unsafe for consumption (USDA, 1997). The HACCP team conducts a hazard analysis and identifies appropriate control measures (Corlett, 1998).

Hazard analysis is accomplished in two stages:

(a) Hazard identification based on a review of the origins of possible hazards and (b) hazard evaluation within the frame of the potential significance of each hazard is assessed by considering its severity (referring to health consequences) and its likeliness to occur (based on experience, epidemiological data and available information in the literature). Hazard

analysis is completed by listing all significant hazards associated to each step, and all control measures that can eliminate or control these hazards to an acceptable level (Arvanitoyannis and Hadjicostas, 2001).

If the hazard analysis is not done correctly and the hazards warranting control within the HACCP system are not identified, the plan will not be effective regardless of how well it is followed (Corlett, 1998). Principle 2 Identification of the critical control points (CCPs) in the process. CCPs are steps at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to acceptable levels (Rushing and Ward, 1999).

Principle 2 Determination Critical Control Points:

The HACCP team should identify the steps in the production process which are essential for the elimination or significant reduction of the identified hazards from Principle 1. These CCPs are identified through the use of the decision tree. A CCP should be a quantifiable procedure in order for measurable limits and monitoring to be achievable in Principles 3 and 4.It is not possible to find CCPs for all types of products and hazards. Especially in low-processed products such as fresh meat, there is almost no site at which microbial hazards can be eliminated. Thus, only hygiene concepts using the basic HACCP methodology can be developed (Upmann and Jacob, 2004).

Principle 3 Establishment critical limit(s) for preventive measures associated with each identified CCP:

Once the CCPs have been determined, a critical limit or the amount of acceptable deviation has to be established for each CCP. Critical limits for CCPs are expressed as numbers or specific parameters on visual observation, such as time/temperature, humidity, water activity, pH, salt concentration and chlorine level (USDA, 1997; Corlett, 1998).

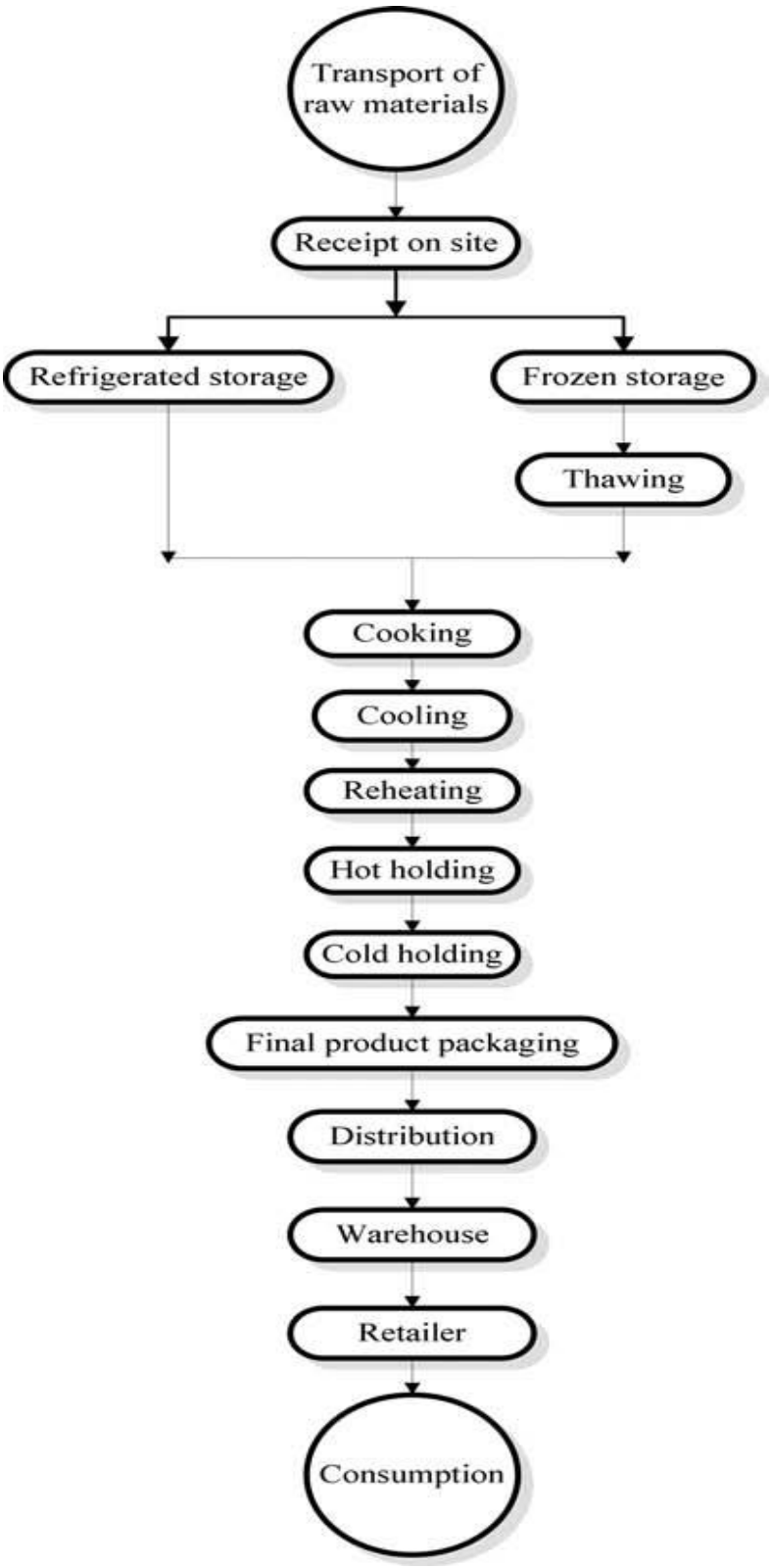

Figure 2: The seven principles involved in developing and operating a HACCP program (Arvanitoyannis, 2009).

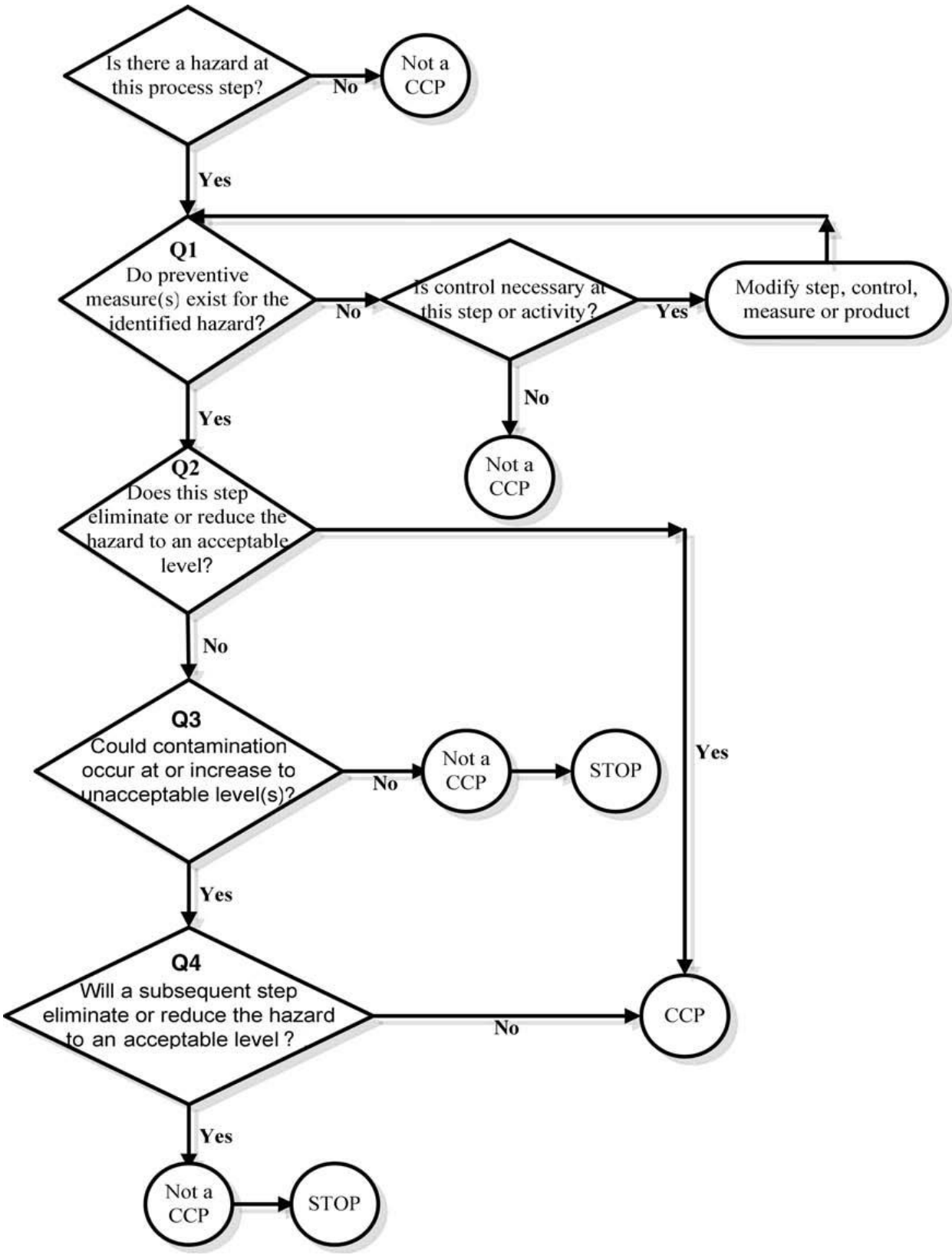

Figure 3**:** Process step CCP decision tree. (Adapted from Corlett, 1998).

Principle 5 Establishment of corrective actions to be taken when monitoring indicates that a particular CCP is not under control:

The regulation defines corrective action as 'procedures to be followed when a deviation occurs'. A deviation is a failure to meet a critical limit (USDA, 1997).

The purpose of corrective actions is:

1. To adjust the process, such as cooking temperatures or cooling rates to maintain control or prevent a deviation
2. To correct the cause of the deviation
3. To re-establish control over the process and CCP
4. To determine the safety and proper disposition of the food being produced while a defect was occurring.
5. To maintain records of corrective actions (Ropkins and Beck, 2000).

All corrective actions cannot be anticipated. An unlisted corrective action should be incorporated into the corrective action document. The corrective action will consist of the decision regarding disposal of non-complying material, correcting the cause of deviation, demonstrating that CCP is once again in control, and, finally, maintaining records of the corrective action (Deodhar, 1999).

Principle 6 Establishment of procedures for verification to confirm that the HACCP system is working effectively.

Verification is the application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan (FAO/WHO, 2001). The verification typically consists of two phases. First, verification that the critical limits established for CCPs will prevent, eliminate or reduce hazards to acceptable limits. Second, verification that the overall HACCP plan is functioning effectively. Once critical limits at each CCP are met, minimal sampling of the final product is needed.

Basic verification procedures include the following:

1. Initiation of appropriate verification inspection schedules
2. Review of HACCP plan for completeness
3. Confirmation of the accuracy of flow diagram
4. Review of CCP records
5. Review of records for deviations and corrective actions
6. Review of critical limits to verify if they are adequate to control significant hazards
7. Validation of the HACCP plan, including on-site review
8. Review of the modifications made to the HACCP plan
9. A random sample collection and analysis
10. Visual inspection of food production operations to determine that CCPs are under control
11. A review of departures from critical limits and how they were corrected (Corlett, 1998).

Principle 7 Establishment of documentation concerning all procedures and records appropriate to these principles and their application.

The level of documentation required will depend upon the needs and the complexity of the food business. In a small business, a simple log book or diary may be all that is needed. In a bigger or more complicated business, more detailed or formal documentation will be necessary. Record keeping and documentation systems should meet the needs of the business and be adequate to show that the food safety program is working. The HACCP will incorporate documents such as the following:

1. The HACCP plan
2. Hazard analysis
3. CCP determinations
4. CCP monitoring sheets

5. Corrective actions
6. Audit records
7. HACCP team meeting minutes
8. Calibration records (Slatter, 2003).

2.2.2.3 The 12 stages of the HACCP plan

Brennan and Grandison, (2011) mention the principles of HACCP as follows:

Developing a HACCP System

In order to develop a HACCP system, a food company applies the Codex HACCP principles to its operations. This is most easily achieved using the following logic sequence (Box), also proposed by Codex.

Logic sequence of HACCP plan

This approach works well in manufacturing operations and normally includes, as a minimum, the following disciplines:

- Manufacturing or operations personnel who understand the process operations on site.
- Engineering personnel who have knowledge and experience of the equipment and process operations in use on site.

In addition to the above disciplines, it can be helpful to include personnel from the following areas; however the total size of a HACCP team is best kept to 4–6 personnel for ease of management:

- Microbiology;
- supplier/vendor assurance;
- Storage and distribution and Product development.

HACCP plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration.

The HACCP plan is simply the documentation produced that shows how significant hazards will be controlled. quality or technical personnel who

understand the product's technical characteristics regarding hazard control and have up to date information on likely hazards in that sector of the food industry.

Table 7: Logic sequence for the application of HACCP.

Logic sequence for application of HACCP
Step 1 Assemble HACCP team
Step 2 Describe product
Step 3 Identify intended use
Step 4 Construct flow diagram
Step 5 On-Site confirmation of flow digram
Step 6 List all potenzial hazards, conduct a hazard analysis and consider control measures
Step 7 Determine CCPs
Step 8 Establish critical limits for each CCP
Step 9 Establish a monitoring system for each CCP
Step 10 Establish corrective actions
Step 11 Establish verification procedures
Step 12 Establish documentation and record keeping

- Source: Brennan and Grandison (2011).

Table 8: The HACCP principles.

Principle 1 Conduct a hazard analysis.
Principle 2 Determine the critical control points (CCPs).
Principle 3 Establish critical limit(s).
Principle 4 Establish a system to monitor control of the CCP.
Principle 5 Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
Principle 6 Establish procedures for verification to confirm that the HACCP system is working effectively.
Principle 7 Establish documentation concerning all procedures and records appropriate to these principles and their application.

- Source: Brennan and Grandison (2011).

Step 1. Assemble HACCP team HACCP is normally applied by a multidisciplinary team, so that the system is the output of a group with the necessary combined experience and knowledge to take decisions about product safety.

Step 2. Describe product It is important for all members of the HACCP team to understand the background to the product/process that they are about to study. This is achieved by constructing a product description (also known as a process description). The product description is not simply a specification for the product, but rather contains information important to making safety judgments.

The following criteria are normally included:

- Hazard types to be considered.
- Main ingredient groups to be used in the product/process line.
- Main processing technologies.
- Key control measures.
- Intrinsic (recipe) factors.
- Packaging system.
- Start and end points of the study.

The task of constructing a product description helps to familiarize all HACCP team members with the product/process under study. It is normal practice to document the product description and include it with the HACCP plan paperwork. The document is also useful at later stages as a familiarization tool for HACCP system auditors or any personnel who need to gain an understanding of the HACCP plan (Brennan and Grandison, 2011).

Step 3. Identify intended use It is necessary to identify the intended use of the product, including the intended consumer target group, because different uses may involve different hazard considerations and different consumer

groups may have varying susceptibilities to the potential hazards. This information is usually included as part of the product description (Step 2).

Step 4. Construct flow diagram A process flow diagram, outlining all the process activities in the operation being studied, needs to be constructed. This should list all the individual activities in a stepwise manner and should show the interactions of the different activities. The purpose of the process flow diagram is to document the process and provide a foundation for the hazard analysis (Step 5).

Step 5. On site confirmation of flow diagram Since the process flow diagram is used as a tool to structure the hazard analysis, it is important to check and confirm that it is correct. This is done by walking the line and comparing the documented diagram with the actual process activities, noting any changes necessary (Brennan and Grandison, 2011).

This exercise is normally done by members of the HACCP team but could also be done by process line operators. The completed process flow diagram should be signed off as valid by a responsible member of staff, e.g. the HACCP team leader.

Step 6. List all potential hazards; conduct a hazard analysis and consider control measures

Using the process flow diagram, the HACCP team now needs to consider each step in turn and list any potential hazards that might occur. They should then carry out an analysis to identify the significant hazards and identify suitable control measures.

These terms are defined by Codex as follows:

Hazard: a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect;

Hazard Analysis: the process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan;

Control Measure: an action or activity that can be used to prevent, eliminate or reduce a hazard to an acceptable level.

An example of hazard analysis for two steps from the milk process flow diagram is given in Table 13. Note, only one potential hazard has been detailed for each process step – there may be others.

The process of hazard analysis requires the team to transcribe each process activity to a table such as the example given, consider any potential hazards.

Table 9: An example of hazard analysis.

Process step	Hazard and source/cause	Significant hazard? (yes or no)	Control measure
Incoming raw milk	Presence of vegetative pathogens, e.g. *Salmonella*, due to contamination from animal	Yes	Control by pasteurisation step in process
Pasteurisation	Survival of vegetative pathogens, e.g. *Salmonella*, due to incorrect heat process	Yes	Effective heat process (correct time/temperature combination)

- Source: Brennan and Grandison (2011).

Along with their sources or causes and then evaluate their significance. To identify the significant hazards, it is necessary to consider the likelihood of occurrence of the hazard in the type of operation being studied as well as the severity of the potential adverse effect. This may be done using judgment and experience or using a structured 'risk assessment' method, where

different degrees of likelihood and severity are weighted to help with the significance decision. Effective control measures then need to be identified for each significant hazard.

Step 7. Determine CCPs: Critical control points (CCPs) are the points in the process where the hazards must be controlled in order to ensure product safety.

They are defined by Codex as follows:

Critical control point (CCP): a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level (Brennan and Grandison, 2011).

It is important to identify the correct points as CCPs so that resource can be focused on their management during processing. CCPs can be identified using HACCP team knowledge and experience or by using tools such as the Codex CCP decision tree (Figure 4).

Step 8. Establish critical limits for each CCP

Critical limits are the safety limits that must be achieved for each CCP to ensure that the products are safe. As long as the process operates within the critical limits, the products will be safe but if it goes beyond the critical limits then the products made will be potentially unsafe. Critical limits are defined by Codex as follows:

Critical limit: a criterion that separates acceptability from unacceptability.

Critical limits are expressed as absolute values (never a range) and often involve criteria such as temperature and time, pH and acidity, moisture, etc.

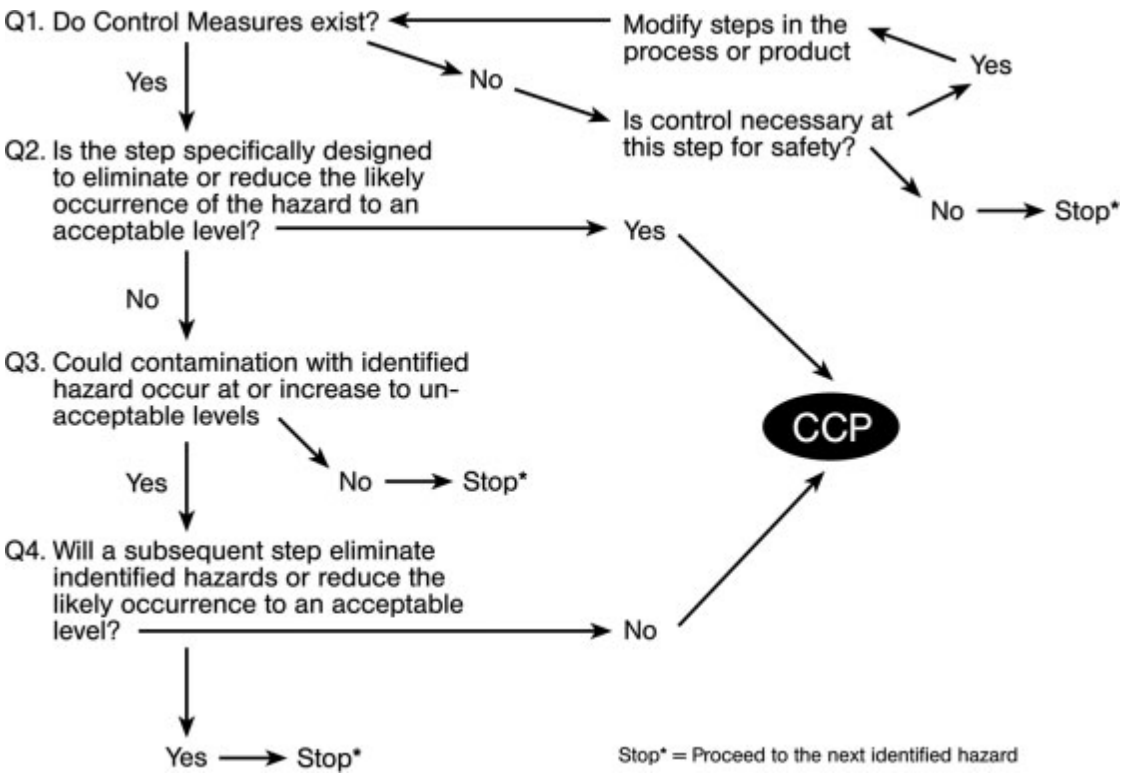

Figure 4: the CCP Decision tree and the process of decision making (Brennan and Grandison, 2011).

Step 9. Establish a monitoring system for each CCP

Monitoring is necessary to demonstrate that the CCPs are being controlled within the appropriate critical limits. Monitoring requirements are specified by the HACCP team during the HACCP study but will usually be done by the process operators when the HACCP plan is implemented in the operation.

Monitoring: the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control. Monitoring should be defined in terms of the monitoring activity itself, along with the frequency and responsibility for doing the task.

Monitoring should be defined in terms of the monitoring activity itself, along with the frequency and responsibility for doing the task (Brennan and Grandison, 2011).

Step 10. Establish corrective actions

Corrective action needs to be taken where monitoring shows that there is a deviation from a defined critical limit. Corrective actions will deal with the material produced while the process is out of control and will also bring the process back under control. Corrective action: any action to be taken when the results of monitoring at the CCP indicate a loss of control; Codex. As for monitoring, the corrective action procedures and responsibility need to be identified by the HACCP team during the HACCP study, but will be implemented by the appropriate operations personnel if deviation occurs.

Step 11. Establish verification procedures: The HACCP team needs to consider how to determine if the HACCP system is valid and working effectively over time. Verification procedures are the methods that will be used to demonstrate compliance and verification is defined by Codex as:

Verification; the application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan. Commonly used verification procedures include:

- HACCP audits;
- Review of CCP monitoring records;
- Validity assessment of HACCP plan elements;
- Product testing – microbiological and chemical;
- Review of deviations, including product disposition and customer complaints.

Table 10: Example of CCP control.

Process step	Hazard	Control measure	Critical Limit	Monitoring			Corrective Action	
				Procedure	Frequency	Responsi-bility	Activity	Responsi-bility
Pasteur-isation	Survival of vege-tative pathogens, e.g. *Salmonella*	Correct temperature and time regime: effective heat process	71.7°C for 15 s	Chart recorder: visual check and sign off	Each batch	Pasteuriser operator	Report to supervisor, contact QA and discuss; ensure divert working correctly; if not, dump/ re-process	Pasteuriser operator, production supervisor, QA manager, plant engineer
				Check auto-divert function	Daily at start up and shutdown	Pasteuriser operator	Hold product until correct heat process verified; dump/ reprocess if not	Pasteuriser operator, production supervisor, QA man-ager, plant engineer

- **Source:** Brennan and Grandison (2011).

Step 12. Establish documentation and record keeping

It is important to document the HACCP system and to keep adequate records. The HACCP plan will form a key part of the documentation, outlining the CCPs and their management procedures (critical limits, monitoring, and corrective action). It is also necessary to keep documentation describing how the HACCP plan was developed, i.e. the hazard analysis, CCP determination and critical limit identification processes. When the HACCP plan is implemented in the operation, records will be kept on an ongoing basis. Essential records include:

- CCP monitoring records.
- Records of corrective actions associated with critical limit deviation.

- Records of verification activities.
- Records of modifications to processes and the HACCP plans.

2.2.2.4 Advantages and disadvantages of HACCP

Food companies that have had effective sanitation and HACCP programs have a number of positive operating characteristics that distinguish them from companies that do not have these programs (Corlett, 1998).

- Application of HACCP system throughout the food chain from the primary producer to the consumer.
- More effective use of resources, savings and more timely response to food safety problems.
- Internationally recognized.
- The application of HACCP systems can promote international trade by increasing confidence in food safety.
- The HACCP system allows for the identification of conceivable, reasonably expected hazards, even where failures have not previously been experienced. It is therefore particularly useful for new operations.
- Staff and business owners gain confidence and are better equipped for informed discussion on food safety measures with food inspectors, third-party auditors, consultants, trading partners, consumers and others.
- The development of a HACCP system can lead to improved education and awareness of staff working in SLDBs and staff members are empowered when their input is sought and valued.
- The HACCP system has strengthened the regulatory approach to food safety by providing food control authorities with an opportunity to revisit their method of food inspection and the training provided to food inspectors.

- More focused control on processes critical to food safety, with the flexibility to accommodate additional changes in production, quality or other specific measures, e.g. control of allergens or emerging pathogens.
- Demonstrable improvements to food quality and safety standards, thereby reducing the potential for foodborne disease, customer complaints, wastage and damage to the reputation of the business (Motarjemi, 2000).

Disadvantages of HACCP

- Resource-intensive during development, unless supported by extensive structure of trade associations or other industry groupings.
- Needs to be validated for effectiveness.
- Difficult to anticipate all hazards introduced by subtle variations on seemingly standard processes thus needs constant vigilance and updating.
- Element of technical knowledge required to adopt them.
- Perceived complexity and bureaucracy – many smaller businesses regard HACCP as complicated and bureaucratic.
- Lack of knowledge and adequate training – many small businesses remain unaware of HACCP or lack sufficient in-house knowledge and training about the risks associated with their procedures to put in place or maintain effective HACCP-based controls.
- The costs of ongoing training against a backdrop of high staff turnover, typical in the industry, can also be prohibitive for many smaller food businesses (FAO/WHO, 2006).

2.3 ISO 22000:2005

2.3.1 History of ISO 22000:2005

In 2001, ISO started the development of an auditable standard, which further defines HACCP's role in FSMS and culminated in the newly formed ISO 22000.

The publication of ISO 22000 was complemented by an ISO Technical Specification (ISO/TS 22004) giving guidance on the implementation of the standard, with a particular emphasis on small- and medium-sized enterprises.

Working Group 8 (WG 8) on FSMS prepared ISO 22000 and ISO/TS 22004, which were both published in 2005 (FAO/WHO, 2007). Another Technical Specification (ISO/TS 22003) was also published explaining certification requirements applicable when third-party certification is used (Frost, 2005). The Draft International Standard ISO/DIS 22000 was issued on 3 June 2004. The deadline for comments was 3 November 2004. ISO 22000 was expected to be available as an International Standard in 2005 (Faergemand and Jespersen, 2004). ISO circulated the final draft of the standard to the national standard bodies that make up its membership for a 2-month voting period, ending on 5 July 2005. The standard can be applied on its own, or in combination with other management system standards such as ISO 9001:2000, with or without independent (third party) certification of conformity (Frost, 2005). The working group that developed ISO 22000 has representatives from 14 countries and input from 13 others representing all continents. In the working group, there are also representatives from organizations such as the Codex Alimentarius, the Global Food Safety Initiative (GFSI) and the Confederation of Food and Drink Industries of the EU (CIAA) (Arvanitoyannis, 2009).

Development of ISO 22000 is given in Table below

Table 11: The development of ISO 22000

Date	Event
2001	Development of the standard
3 June 2004	Draft International Standard ISO/DIS 22000
3 November 2004	Deadline for comments on the draft International Standard
5 July 2005	Final draft of the International Standard

- Source: Arvanitoyannis (2009).

The design and implementation of an organization's food safety management system are influenced by varying factors, in particular food safety hazards, the products provided, the processes employed and the size and structure of the organization. This Technical Specification provides guidance on the use of ISO 22000, which is based on the principles of HACCP as described by the Codex Alimentarius Commission and is designed to be applied together with relevant standards published by that organization (ISO 22000:2005). ISO 22000 will dynamically combine the HACCP principles and application steps with PRPs, using the hazard analysis to determine the strategy to be used to ensure hazard control by combining the PRPs and the HACCP plan (Faergemand and Jespersen, 2004).

2.3.2 Application of ISO 22000:2005

Arvanitoyannis (2009) mentions in his book that, ISO 22000:2005 applies to all organizations, regardless of their size, that impact the food chain. The standard was drafted to serve the needs of not just food producers and manufacturers, but also virtually every other organization that participates in the food supply chain. ISO 22000 is written with a structure compatible to other management system standards in the light of ISO 9001:2000. (Applying ISO 15161 as guideline) while combining HACCP MS/Codex HACCP.

Direct or indirect organizations which can be certified with ISO 22000 standard are the following:

(a) Direct organizations

Farmers, Harvesters, bait producers, food component producers, food producers, food sellers, food services, ready-made food companies and organizations which service cleaning, sanitizing, carriers, storage distribution etc.

(b) Indirect organizations'

Producers of equipment, package materials, ingredients and additives, organizations' etc. producing other elements which contact with food (Pillay and Muliyil, 2005).

2.3.3 Benefits of ISO 22000:2005

Adopting the ISO 22000 standard provides the company with competitive efficiencies worldwide. With registration to ISO 22000, the ensuing advantages are:

- Incorporation of legal and regulatory requirements relating to food safety including HACCP systems.
- A uniformly auditable standard.
- A drive for continuous improvement.
- Improved internal and external communications.
- Improved documentation.
- Improved compliance with hygiene regulations.
- Improved food safety hazard control.
- Easy to understand, apply and recognize.
- Facilitates traceability and clear communication across the supply chain.
- Clear responsibilities and authorities agreed for all staff.
- Resource optimization (internally and along the food chain).
- Valid basis for taking decisions.
- Provides a framework for third-party certification.
- Can be applied independently.
- Allow small and/or less developed organizations' to implement an externally developed system.
- Speeds and simplifies processes, increases efficiency and reduces costs without compromising existing or other quality or management systems.

- Applicable to all organizations' in the global food supply chain.
- The structure aligns with the management system clauses of ISO 9001 and ISO 14001.
- All control measures are subjected to hazard analysis.
- Better planning – less post-process verification systematic management of PRPs.
- A systematic and proactive approach to identification of food safety hazards and development and implementation of control measures.
- Enables streamlined communication and collaboration for quicker, more informed decision making about hazards with supply chain partners.
- Increased international acceptance of food products.
- Reduces risk of product/service liability claims.
- Ensures safety of food products.
- Greater health protection.
- Job productivity and satisfaction of employees are increased.
- Employees become conscious about hygiene and food safety.
- Can be applied by all manufacturers and participants in the entire food chain supply.
- Food wastes (food decaying etc.) fees decrease to minimum.
- Work environment gets better.
- It is a trusted system which was confirmed by FAO/WHO (Arvanitoyannis, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of samples

Three soft drinks factories were chosen according to their quality system and the similarity of their products to be tested (orange flavoured carbonated soft drink). The three plants will be referred to as "Plant A" Located in Khartoum North (implements ISO 22000:2005) "Plant B" located in Khartoum and (implements Good Manufacturing Practice) and finally "Plant C" located in Omdurman (doesn't implement quality system). Samples were obtained on the same day of production and went through microbial analysis using the filtration method.

Three random samples were taken for each test. Then sample were kept in their original polyethylene terephthalate bottles at room temperature (27°C) and then kept refrigerated until needed for the different investigations.

3.1.2 Chemicals, Media and equipment

- Iso propyl alcohol 70% from china.
- Distilled water.

Media:

Media used were purchased with the trade mark – Dr.Moller and Schmelzt ™ – Germany in the form of nutrient pads detailed below:

- **Plate count-NPS** used for plate count.
- **Malt extract-NPS** a selective medium for yeasts and molds.
- **Colichrome -NPS** a selective for the rapid quantitative detection of *E. coli* and coliform bacteria by optical differentiation.
- **Orange Serum-NPS** which is selective for acidophilic and acid tolerant microbes in beverage and food.

Note: acidtolerant microbes' strains targeted by medium Orange Serum-NPS are:

Aspergillus brasiliensis

Saccharomyces cerevisiae

Zygosaccharomyces rouxii

Devices and equipment's:

- Incubator – IB- 05G/ JEIO tech. Korea.
- Heating Incubator -DHP-9052/ RT 5 to 65 degrees. Germany.
- Laminar Flow, Clean Bench IBC – 11E / JEIO tech. Korea.
- Bincolor Microscope B 130 - Optika. Italy
- Drying Oven- DHP- 9050 A. Germany
- Glassware of Simtik- Czech Republic and Iso Lab- Germany.
- Autoclave. Italy
- Stainless Steel Manifold. 3 funnels. Sartorius stedim biotech- Germany.

Gridded Membrane filters:

- Coliform bacteria and E. coli; white gridded ME 25/21 ST – 0.45 µm.
- Plate count. ; Green gridded 20/41 ST – 0.45 µm.
- Acid tolerant microbes; green gridded 25/41 ST – 0.45 µm.
- Yeast; black gridded 26/31 ST – 0.6 µm.
- Mold; black gridded 26/31 ST – 0.6 µm.

3.2 Methods

Microbiological analysis was determined according to the membrane filtration method which is based on the standard method of the Association of Official Analytical Chemists (AOAC, 1990; ISTB, 2008; Rice and Bridgewater, 2012; Abeyta et al., 2015) detailed as in the following:

3.2.1 Microbiological analysis of samples:

Principal: Hydrophobic grid membrane filter (HGMF) uses membrane filter imprinted with hydrophobic material in grid pattern. Hydrophobic lines act as barriers to spread of colonies, thereby dividing membrane filter into separated compartments of equal and known size. Numbers of squares occupied by colonies is enumerated and converted to most probable number values of organisms.

All equipment's were disinfected; forceps, pipette and glass-ware were disinfected using autoclave. All surfaces were wiped with 70% ethanol solution and ultra violet light was used in clean chamber device. Flame was on during experiment. Protective gloves, clothes and hair net were also worn. PET bottles were washed and wiped with 70% ethanol solution prior examination.

3.2.1.1 Total plate count:

Plate count was determined using the membrane filtration method according to the standard method of the Association of Official Analytical Chemists (AOAC, 1990) where a pack of ten was opened and a Petri dish was removed containing a nutrient pad –medium.

3 – 3.5 ml sterile, distilled or demineralized water was added to the -plate count-NPS- nutrient pad in the Petri dish. Moisture level is optimal, if an excess ring of liquid was clearly visible.

Sealed envelope was opened, **0**.45 µm green, gridded membrane filter 20/41 ST membrane filter was removed with sterile tweezers, the membrane filter was placed on top of the frit of the filter holder and the filter funnel was put on.

Sample was filtered by switching on the pump.

The membrane filter was then aseptically and carefully removed from the frit with a sterile tweezers and placed on the prepared nutrient pad without catching air bubbles.

Petri dish was incubated with the lid facing upwards for 48 hours ± 3 at an incubation temperature of 35 °C ± 1°C.

3.2.1.2 Total yeasts and molds:

Yeasts and molds were determined using the membrane filtration method according to the standard method of the Association of Official Analytical Chemists (AOAC, 1990) where a pack of ten was opened and a Petri dish was removed containing a nutrient pad -medium

3 – 3.5 ml sterile, distilled or demineralized water was added to medium selective -"Malt Extract-NPS" which is selective for the detection of yeast and mold from beverages - nutrient pad in the Petri dish. Moisture level is optimal, if an excess ring of liquid was clearly visible.

Sealed envelope was opened, 0.6 µm black, gridded membrane filter 26/31 ST – membrane filter was removed with sterile tweezers, the membrane filter was placed on top of the frit of the filter holder and the filter funnel was put on.

Sample was filtered by switching on the pump.

The membrane filter was then aseptically and carefully removed from the frit with a sterile tweezers and placed on the prepared nutrient pad without catching air bubbles.

Petri dish was incubated with the lid facing upwards for 120 hours ± 3 incubation periods at 25 °C ± 1°C.

3.2.1.3 Total coliform bacteria:

Total coliform bacteria were determined using the Membrane filtration method according to the standard method of the Association of Official

Analytical Chemists (AOAC, 1990) where a pack of ten was opened and a Petri dish was removed containing a nutrient pad -medium

3 – 3.5 ml sterile, distilled or demineralized water was added to medium selective “Colichrome -NPS” which is selective for the rapid quantitative detection of E. coli and coliform bacteria by optical differentiation within 24 hours- nutrient pad in the Petri dish. Moisture level is optimal, if an excess ring of liquid was clearly visible.

Sealed envelope was opened, 0.45 µm white, gridded membrane filter ME 25/21 ST – membrane filter was removed with sterile tweezers, the membrane filter was placed on top of the frit of the filter holder and the filter funnel was put on.

Sample was filtered by switching on the pump.

The membrane filter was then aseptically and carefully removed from the frit with a sterile tweezers and placed on the prepared nutrient pad without catching air bubbles.

It was then incubated the Petri dish with the lid facing upwards for With 24 hours ± 3 incubation periods at 35 °C ± 1°C.

Note: E.coli from blue and coliform bacteria red colonies**.**

3.2.1.4 Acid tolerant microbes:

Acid tolerant microbes were determined using the membrane filtration method according to the standard method of the Association of Official Analytical Chemists (AOAC, 1990) where a pack of ten was opened and a Petri dish was removed containing a nutrient pad –medium.

3 – 3.5 ml sterile, distilled or demineralized water was added to medium selective “Orange Serum-NPS” which is selective for acidophilic and acidotolerant microbes in beverage and food.- nutrient pad in the Petri dish. Moisture level is optimal, if an excess ring of liquid was clearly visible**.**

Sealed envelope was opened, 0.45 µm green, gridded membrane filter 25/41 ST – membrane filter was removed with sterile tweezers, the membrane filter was placed on top of the frit of the filter holder and the filter funnel was put on.

Sample was filtered by switching on the pump.

The membrane filter was then aseptically and carefully removed from the frit with a sterile tweezers and placed on the prepared nutrient pad without catching air bubbles.

It was then incubated the Petri dish with the lid facing upwards for With 120 hours ± 3 incubation periods at 25 °C ± 1°C.

Note: Anaerobic incubation also meditates growth of more demanding lactobacilli.

After incubation, promptly all colonies on the membrane surface were counted. If it's impossible to count immediately after incubation, plates are stored at approximately 4°C for a period of not more than 24 hours. If the number of colonies per membrane surface is less than 100 all colonies are Counted and recorded as per volume of sample. If the number of colonies is greater than 100 but less than 300, membrane was divided into 4 quadrants and the number of colonies in one quadrant is counted then multiplied by 4 and recorded and reported as volume of sample. If the total number of colonies was greater than 300, colonies are then recorded and reported as TNTC per volume of sample.

3.2.2 Verification of HACCP plan of plant A

Verification of the seven principles of the HACCP plan was made using the PrimusGFS Audit HACCP (Module 3) Guidelines, Which is based on the Food and drugs administration guidance regulations of HACCP. The organization will determine the need for a HACCP program by performing a documented hazard analysis of all steps of each process (PrimusGFS, 2014).

The three plants were approached contacting the quality control in charge who in the three plant assisted and facilitated the survey with a number of difficulties since not all plants welcome audit like activities.

PrimusGFS Audit HACCP (Module 3) was followed carefully in each of the three plants and was easy to use for both the auditor and the auditee.

All questioned were asked and answered and score has been given accordingly to each of the three plants.

PrimusGFS gives score based on standard given questions with a corresponding score to each question evaluating the HACCP pimples as follows:

1. **Has a documented hazard analysis for the process been conducted, showing the various types of hazard, their likelihood of occurrence and their associated severity?**

   Total compliance (15 points): A hazard analysis identifies and evaluates hazards, and determines if control measures are in place to prevent, eliminate or reduce the food safety hazard to an acceptable level. A detailed hazard analysis for each process flow should have been conducted and documented in order to prove that a proper hazard analysis was conducted. Note, if there are errors in the process flow it is likely there will also be errors in the hazard analysis. At each step of the process, from raw material receipt and storage, through processing and packing, storage and distribution the hazard analysis should look at the severity and likelihood of all potential food safety hazards that may be reasonably expected to occur in terms of specific biological, chemical and physical or other issues.

   Minor deficiency (10 points) if:

   • Single/isolated instance(s) of errors or omissions on the hazard analysis chart(s).

Major deficiency (5 point) if:

• Numerous instance(s) of errors or omissions on the hazard analysis chart(s)

• In an operation with multiple products/processes that are not similar, one hazard analysis chart is not available.

Non-compliance (0 points) if:

• Multiple systematic errors on the hazard analysis chart(s).

• No process hazard analysis chart(s).

• In an operation with multiple products/processes that are not similar, more than one hazard analysis chart is not available.

2. **Have CCPs been developed?**

Total compliance (15 points): If answer is YES, continue with next question. If answer is NO, the rest of "Module 3 HACCP" is not applicable.

Total points (0): The identification of a CCP in the process will require the development of the criteria for managing it and the execution of the necessary activities in the production line. Not having a CCP in the process means that these steps are not applicable for the operation.

3. **Have CCP decisions been made with documented justifications and where CCPs are noted have they been developed to control the hazards identified in the hazard analysis step?**

Total compliance (15 points): CCP decisions should be properly justified with supporting documents and evidence. The CCPs defined in the hazard analysis should be developed to define in detail the parameters involved, and monitoring requirements to control the hazard(s).

Minor deficiency (10 points) if:

• Single fault in the logic or justification of one CCP decision.

• Single CCP developed that does not meet the criteria for a CCP.

Major deficiency (5 point) if:

• More than one fault in the logic or justification of the CCP decisions.

• More than one CCP developed that does not meet the criteria for a CCP.

• One (where there are multiple) CCP has been omitted.

Non-compliance (0 points) if:

• No CCP's have been developed in the hazard analysis step even though clearly CCPs did exist.

• More than one CCP has been omitted in a plan where there should be multiple CCPs.

• A single CCP has been omitted in a plan where there is a single CCP.

4. **Have CCP critical control limits been established with support of relevant sources of information or by validation documentation?**

Total confirmation (15 points): All CCP's should be supported by validation documentation showing that the critical control limits (CCL) are scientifically derived and meet any relevant legal requirements. Where publicly available validation is not available, the auditee should have performed validation studies to support their stated critical control limits. For example, ORP limits for chlorinated recycled water systems could be stated in research papers and State documentation e.g. Leafy Greens Marketing Agreement.

Another example, metal detection limits could be supported by validation studies that show that smallest test probes possible were used and meet the FDA guidelines

Minor deficiency (10 points) if:

• Single/isolated instance(s) of omissions or incorrect CCL validation details.

Major deficiency (5point) if:

• Numerous instances of omissions or incorrect CCL validation details.

Non-compliance (0 points) if:

• There is no documentation to support CCP critical control limits.

• Systematic omissions or incorrect CCL validation details.

5. **Have monitoring requirements and frequencies been determined for the CCPs?**

Total compliance (15 points): Monitoring requirements and frequencies should have been determined for the CCPs. Where monitoring is not continuous, the type and frequency of monitoring should be sufficient to ensure the CCP is under control. Frequency should be specified; "as needed" is not accepted as a stated frequency. The requirements i.e. what is to be done should be specified on the HACCP chart. Requirements should include the critical control limits (CCL's) i.e. the maximum and/or minimum parameters of what is being monitored e.g. with a metal detector, the sensitivity of the detector setting should be stated and size/type of test pieces used, or with an anti-microbial the minimum concentration required should be stated. Other CCLs may include temperature parameters, pH, flow rates, dwell times, etc.

Minor deficiency (10 points) if:

• Single/isolated instance(s) of omissions or errors in the monitoring requirements.

• Single/isolated instance(s) of omissions or errors in the frequency details.

Major deficiency (5 point) if:

• Numerous instances of omissions or errors in the monitoring requirements.

• Numerous instances of omissions or errors in the frequency details.

• A single CCP (where there are multiple CCP's) is lacking monitoring requirements or frequency details.

Non-compliance (0 points) if:

• More than one CCP is lacking monitoring requirements or frequency details where there are multiple CCP's in a plan.

• A single CCP is lacking monitoring requirements or frequency details in a plan where there is a single CCP.

6. **Have specific responsibilities been assigned for the monitoring, recording and corrective action management of each CCP?**

Total compliance (10 points): Specific responsibilities should be assigned for the monitoring, recording and corrective actions of each CCP. If CCP records are not being completed properly, this may be an indication that the CCPs have not been assigned correctly. The responsibility should be clearly indicated on the HACCP chart by at least naming the function e.g. QA Department, who are responsible for monitoring, recording and executing corrective action related to an individual CCP.

Minor deficiency (7 points) if:

• Single instance of a CCP not being assigned (to either a person or group), where there are multiple CCPs.

Major deficiency (3 point) if:

• Numerous instances of a CCP not being assigned (to either a person or group), where there are multiple CCPs.

Non-compliance (0 points) if:

• No CCPs have been assigned to either a person or group.

7. **Have standard operating procedures (SOPs) been created for the monitoring process of the CCPs, which would include how to carry out the monitoring activities?**

Total compliance (5 points): Clear and simple standard operating instructions (SOPs) should be written for each CCP monitoring process – this expands in detail the CCP monitoring in the form of work instructions. These SOPs must match what is written in

the HACCP plan. These SOPs can be used for training and as reference tools.

Minor deficiency (3 points) if:

• Single/isolated instance(s) of errors and omissions within the CCP SOPs.

Major deficiency (1 point) if:

• Numerous instances of errors and omissions within the CCP SOPs.

• Single instance of a CCP SOP not being created in a system where there are multiple CCPs.

Non-compliance (0 points) if:

• CCP SOP(s) has/have not been created.

• CCP SOP(s) do not reflect at all the reality of what is being performed in the operation.

8. **Have corrective action procedures for the CCPs been established, including a detailed action plan for operators to follow if the limits are not met and plans to adjust the process back into control?**

Total compliance (15 points): The corrective action details should note the critical control limit issue that has occurred, what corrective actions were carried out, including what happened to potentially affected product and also how the process was "repaired" or "amended" in order to get the process back to the required control level. The HACCP plan corrective action sections should state where the corrective action details are to be recorded. Where required, preventative measures should also be recorded.

Corrective actions should ensure that the CCP has been brought under control and require that a review is conducted in order to prevent a recurrence of the situation.

Minor deficiency (10 points) if:

• Any one of the above criteria is missing in the corrective action plan details.

• Single/isolated instance(s) of omission or errors in the corrective action details.

Major deficiency (5 point) if:

• Two of the above criteria are missing in the corrective action plan details.

• Numerous instances of omission or errors in the corrective action details.

Non-compliance (0 points) if:

• More than two of the above criteria are missing in the corrective action plan details.

• Systematic errors in corrective action plan details.

9. Have recording templates (recording forms) been developed for monitoring the CCPs?

Total compliance (10 points): Monitoring records should have been designed to record the CCPs that have been identified. The records should match the details as noted in the HACCP Plan and have CCPs identified by name and number, what is being measured, the frequency of the measurement, the critical control limit, the operating limit, the responsible person(s) or team and the corrective action(s) required in the case of measurements not in compliance. Recording forms should have a specific document code as part of the document control program (1.02.01). The records ideally show the CCP parameters (not a scoring issue).

Minor deficiency (7 points) if:

• Single/isolated instance(s) of a record(s) having been developed but does/do not match the details in the HACCP plan i.e. information or requirements on the recording template that does not match what is noted in the plan.

Major deficiency (3 point) if:

• Numerous instances of a record(s) having been developed but do not match the details in the

HACCP plan i.e. information or requirements on the recording template that does not match what is noted in the plan.

Non-compliance (0 points) if:

• Systematic failure of record(s) that have been developed to match the details in the HACCP plan i.e. information or requirements on the recording template that does not match what is noted in the plan.

• Single instance where a CCP has been created but a record for the monitoring data has not been developed.

10.Have verification plans and schedules been developed for each CCP?

Total compliance (10 points): Verification activities related to each CCP on the HACCP chart should be clearly detailed. Verification activities should include a verification of the CCP monitoring records by a HACCP trained supervisor or manager, checking that the CCP monitoring records have been completed in a proper and timely manner and including any corrective action work. Note, a CCP operator cannot verify their own work. Verification activities might include microbial testing, customer complaints and any other information that CCPs might help generate. Verification information might help improve and develop the HACCP program, but should show that the plan is being implemented correctly, is controlling the risk to an acceptable level (or eliminating the risk) and where this is not the case, this should be indicated on the verification paperwork along with corrective action details (e.g. reviewing a CCP, a process flow, a hazard analysis step, etc.).

Minor deficiency (7 points) if:

• Single/isolated instance(s) of errors or omissions in the verification details on the plan.

Major deficiency (3 point) if:

• Numerous instances of errors or omissions in the verification details on the plan

• Single instance in a plan with multiple CCPs where verification details have not been noted.

Non-compliance (0 points) if:

• No verification plans have been developed for any CCP.

11. Are changes in the process, equipment, ingredients etc., causing timely reviews of HACCP systems, including hazard analysis, CCP decisions, CCP records and staff training?

Total compliance (10 points): When any changes are made to the process, equipment, ingredients, etc., all HACCP systems should be reviewed and the HACCP coordinator should inform all employees involved. Re-training or educational sessions may be necessary. Look for evidence of plan change, review of hazard analysis, CCP decisions, CCP records and check to see if key operators were informed/retrained. All changes should be dated. If no changes have occurred, quiz the auditee how they would communicate the changes, if they happened in the future. Records of any re-training should be available.

Minor deficiency (7 points) if:

• Single/isolated instance(s) of omissions or incorrect data in the records.

• Single/isolated instance(s) of required employees e.g. CCP operators, supervisors etc. not being informed about changes to the HACCP plan.

Major deficiency (3 point) if:

• Numerous instances of omissions or incorrect data in the records.

• Numerous instances of required employees e.g. CCP operators, supervisors etc. not being informed about changes to the HACCP plan.

Non-compliance (0 points) if:

• Changes to the process, equipment, ingredients, etc., have taken place but there has been no review of HACCP systems.

• HACCP plan has been changed and none of the required employees were informed.

• Re-training records have not been maintained.

12. Is there evidence recorded for HACCP training to all plant employees, including training for CCP operators?

Total compliance (10 points): All site employees should receive basic HACCP overview training i.e. what HACCP, the 7 principles, is and what are the CCPs on site. Basic training might form part of the new hire orientation package. CCP operators should be specially trained for their function(s) and include the operations they are responsible for. Senior management should also receive training (HACCP requires "buy in" from all levels). Records of training should be kept and also certificates where relevant. All employees should be trained to understand the principles of HACCP and the plan implemented in the facility. Training should be scheduled on a regular basis and documented. The training should be tailored to the people and their positions within the company.

Minor deficiency (7 points) if:

• Not all plant employees are trained in HACCP (but all key operators and majority of employees have been trained).

• Senior management has not received HACCP training.

• Single/isolated instance(s) of omissions or incorrect data in the records.

Major deficiency (3 point) if:

• HACCP coordinator has not completed a certified HACCP training course.

• CCP operators have not been trained in their specific functions.

• Numerous instances of omissions or incorrect data in the records.

Non-compliance (0 points) if:

• No formal training session developed for employees.

• No records of training being maintained.

Given scores are detailed in table below for each question:

Table 12: Scores for HACCP verification.

Possible Answer	Possible Points for the question			
Total compliance	15 points	10 Points	5 Points	3 Points
Minor deficiency	10 points	7 Points	3 Points	2 Points
Major deficiency	5 points	3 Points	1 Points	1 Point
Non-compliance	0 points	0 points	0 Points	0 Points
Not applicable	0 points	0 points	0 Points	0 Points

- Source: PrimusGSF (2014).

3.2.3 Statistical analysis

Statistical analysis was conducted using the experiment Model: Two Factor Completely Randomized Design using the statistical analysis system (SAS).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Microbiological profile of carbonated soft drinks from the three factories (A, B and C):

Table (13) below shows that the TBC of samples obtained from plant A and B did not at change (P≤0.05) in each of the sample collection days. TBC of samples from plant C increased significantly (P≤0.05) up to day 2 of sample collection, then the increase was insignificant.

At any sample collection day, samples form plant C had way higher counts than those from plant A or B.

Regardless to the sample collection day, sample from plant C had a significantly higher counts (33.33 cfu\ 100 ml) than those from plant A (1 cfu\ 100 ml) or Plant B (0.67 cfu\ 100 ml).

The observed difference in TBCs' between the three plants could be attributed to the fact that the plants A and B apply quality systems that the third plant (plant C) doesn't apply.

It is sensible to mention that these results did not comply with the Sudanese standard SSMO (250/2007), (SSMO, 2007) but they complied with the relevant gulf standard for non-alcoholic carbonated beverages (GSO, 1998). UAE.S/GSO 1016:1998.

Table (14) above shows that Total yeast count of samples obtained from plant A and B did not change (P≤0.05) in each of the sample collection days. Total yeast count of samples from plant C did not change significantly (P≤0.05) at each of the sample collection days.

Table 13: Total bacterial count (cfu/100 ml) of soft drinks obtained from plant A, B and C.

Days	Plant		
	A	B	C
1	1.33^{c} ±0.0	1.33^{c} ±0.0	26.67^{b} ±0.0
2	0.67^{c} ±0.0	0.33^{c} ±0.0	30.67^{a} ±0.0
3	1.00^{c} ±0.0	0.33^{c} ±0.0	33.67^{a} ±0.0
Overall	1.00^{B} ±0.0	0.67^{B} ±0.0	30.33^{A} ±0.0
$Lsd_{0.05}$	3.864*		
SE	1.301		

Values are mean±SD

Mean(s) having different superscript(s) letters are significantly ($P \leq 0.05$) different according to DMRT.

Table 14: Total yeasts (cfu/100 ml) of soft drinks obtained from plant A, B and C.

Days	Plant		
	A	B	C
1	0.00^{b} ±0.0	0.67^{b} ±0.0	20.33^{a} ±0.0
2	0.33^{b} ±0.0	0.33^{b} ±0.0	15.67^{a} ±0.0
3	1.00^{b} ±0.0	0.00^{b} ±0.0	17.33^{a} ±0.0
Overall	0.44^{B} ±0.0	0.33^{B} ±0.0	17.78^{A} ±0.0
$Lsd_{0.05}$	7.521*		
SE	2.531		

Values are mean±SD

Mean(s) having different superscript(s) letters are significantly ($P \leq 0.05$) different according to DMRT.

At any sample collection day, samples form plant C had significantly way higher counts than those from plant A or B.

Regardless to the day of sampling, sample from plant C had a significantly higher counts (17.78 cfu\ 100 ml) than those from plant A (0.44 cfu\ 100 ml) or Plant B (0.33 cfu\ 100 ml).

This observed difference in total yeast count between the three plants could be attributed to the fact that the plants A and B apply quality systems which the third plant (plant C) didn't apply.

It's also noteworthy that these results did not again comply with the Sudanese standard SSMO (250/2007), (SSMO, 2007). In the other hand, Results did comply with the relevant gulf standard for non-alcoholic carbonated beverages (GSO, 1998). UAE.S/GSO 1016:1998.

Table (15) above shows that Total mold count of samples obtained from plant A and B did not change ($P \leq 0.05$) in each of sample collection days. Total mold count of samples from plant C increased throughout the second and the third day of sampling.

However, at any sample collection day, samples form plant C had significantly way higher counts than those from plant A or B.

Regardless to the sample collection day, sample from plant C had a significantly higher counts (1.56 cfu\ 100 ml) than those from plant A (0.22 cfu\ 100 ml) or Plant B (0.11cfu\ 100 ml).

The observed difference in total Mold count between the three plants could be linked to the fact that the plants A and B apply quality systems which the third plant (plant C) didn't apply.

Table 15: Total molds (cfu/100 ml) of soft drinks obtained from plant A, B and C.

Days	Plant		
	A	B	C
1	0.00^{b} ±0.0	0.33^{b} ±0.0	0.67^{ab} ±0.0
2	0.00^{b} ±0.0	0.00^{b} ±0.0	1.67^{ab} ±0.0
3	0.67^{ab} ±0.0	0.00^{b} ±0.0	2.33^{a} ±0.0
Overall	0.22^{B} ±0.0	0.11^{B} ±0.0	1.56^{A} ±0.0
$Lsd_{0.05}$	1.778*		
SE	0.5983		

Values are mean±SD

Mean(s) having different superscript(s) letters are significantly ($P \leq 0.05$) different according to DMRT.

These results did not comply with the Sudanese standard SSMO (250/2007), (SSMO, 2007). However, results did comply with the relevant gulf standard for non-alcoholic carbonated beverages (GSO, 1998). UAE.S/GSO 1016:1998.

Table (16) above shows that the Acid tolerant microbe's count of samples obtained from plant A and B did not change ($P \leq 0.05$) in each of sampling days.

However results of samples collected from plant C kept on decreasing on each sampling day, the decrease was not significant on the second and the third day; and significant on the first sampling day.

At any sample collection day, samples form plant C had significantly way higher counts than those from plant A or B.

Regardless to the sample collection day, sample from plant C had a significantly higher counts (42.56 cfu\ 100 ml) than those from plant A (0.11 cfu\ 100 ml) or Plant B (0.00 cfu\ 100 ml).

The observed difference in total Acid tolerant microbe's count between the three plants could be attributed to fact that the plants A and B apply quality systems which the third plant (plant C) didn't apply.

These results did not comply with the Sudanese standard SSMO (250/2007), (SSMO, 2007). However, Results comply with the relevant gulf standard for non-alcoholic carbonated beverages (GSO, 1998). UAE.S/GSO 1016:1998.

Samples from the three plants (A,B, and C) were free from coliform bacteria, a result that is in accordance with the Sudanese standard SSMO (250/2007), (SSMO, 2007) and gulf standard for non-alcoholic carbonated beverages (GSO, 1998). UAE.S/GSO 1016:1998.

Table 16: Total acid tolerant microbes (cfu/100 ml) of soft drinks obtained from Plant A, B and C.

Days	Plant		
	A	B	C
1	0.00^{c} ±0.0	0.00^{c} ±0.0	51.00^{a} ±0.0
2	0.00^{c} ±0.0	0.00^{c} ±0.0	41.67^{b} ±0.0
3	0.33^{c} ±0.0	0.00^{c} ±0.0	35.00^{b} ±0.00
Overall	0.11^{B} ±0.0	0.00^{B} ±0.0	42.56^{A} ±0.0
$Lsd_{0.05}$			
SE			

Values are mean±SD

Mean(s) having different superscript(s) letters are significantly ($P \leq 0.05$) different according to DMRT.

Nwaiwu and Ibekwe (2006) found that the tulip rubber had the highest yeast count of (45 cfu/100ml) while the vent tube- parts of carbonated soft drinks filling machine- had the least count of (25 cfu/100ml) this indicates that the tulip rubber has the highest potential for contaminating finished products. This result could be a possible explanation of the results of (Plant C) where higher counts of yeast and mold and total count could be associated with poor equipment hygiene which is possible result of absence of quality control system as the lack of equipment hygiene could be a direct result of the negligence to implement a quality system that has an efficient cleaning programs (PRB's), (SSOP) and (GMP).

Oranusi et al. (1994) examined the microbial status of soft drinks in the Nigerian market where microbiological analyses were conducted on 90 samples of soft drinks representing 30 different products commercially available in Nigeria; contaminants were detected in 50% of them. The isolates were mainly saprophytic and nonpathogenic: Bacillus spp. (35%), Lactobacillus spp. (26%), Pediococcus spp. (6%), Staphylococcus epidermidis (6%) and Micrococcus spp. (3%) accounted for the bacterial isolates while Aspergillus niger (6%) and Saccharomyces spp. (16%) accounted for the fungal isolates.

Akond et al. (2009) studied the bacterial contaminants in carbonated soft drinks sold in Bangladesh markets and concluded to suggesting that carbonated soft drinks commercially available in Bangladesh pose substantial risks to public health. A total of 225 carbonated soft drink (CSD) samples from nine brands, from various locations in five metropolitan cities of Bangladesh were examined to determine their bacteriological quality. and the concluded that Most samples were not in compliance with microbiological standards set by organizations like the World Health Organization .

Pseudomonas aeruginosa was the predominant species with an incidence of 95%. Streptococcus spp. and Bacillus stearothermophilus were the next most prevalent with numbers ranging from 6 to 122 and 9 to 105 cfu/100 ml, respectively. Fifty four percent of the samples yielded Salmonella spp. at numbers ranging from 2 to 90 cfu/100 ml. Total coliform (TC) and faecal coliform (FC) counts were found in 68–100% and 76–100% of samples of individual brands, at numbers ranging from 5 to 213 and 3 to 276 cfu/ 100 ml, respectively. According to WHO standards 60–88% of samples from six brands and 32% and 40% of samples from two other brands belonged to the intermediate risk group with FC counts of 100–1000 cfu/100 ml. Heterotrophic plate counts, however, were under the permissible limit in all 225 samples.

4.2 Verification of HACCP plans of plant A

Verification of the seven principles of the HACCP plan for the three plants was conducted using the PrimusGFS Audit HACCP (Module 3) Guidelines. That is based on questions targeting the application of the seven principles on a targeted food plant.

The results of the verification of HACCP plans for the plants A was as follows:

Table 17: Plant (A) HACCP Principles Verification:

HACCP Principles	Questions	Score	Compliance
1- Conduct hazard analysis	1. Has a documented hazard analysis for the process been conducted, showing the various types of hazard, their likelihood of occurrence and their associated severity?	15	Total Compliance
2- Determine Critical control points	2. Have CCPs been developed?	15	Total Compliance
	3. Have CCP decisions been made with documented justifications and where CCPs are noted have they been developed to control the hazards identified in the hazard analysis step?	15	Total Compliance
3- Establish critical limits	4. Have CCP critical control limits been established with support of relevant sources of information or by validation documentation?	15	Total Compliance
4- Establish a system to monitor control of CCPs	5. Have monitoring requirements and frequencies been determined for the CCPs?	15	Total Compliance
	6. Have specific responsibilities been assigned for the monitoring, recording and corrective action management of each CCP?	7	Minor deficiency
	7. Have standard operating procedures (SOPs) been created for the monitoring process of the CCPs, which would include how to carry out the monitoring activities?	3	Minor deficiency
	8. Have recording templates (recording forms) been developed for monitoring the CCPs?	15	Total Compliance
5- Establish corrective actions for deviations from	9. Have corrective action procedures for the CCPs been established, including a detailed action plan for operators to follow if the limits are not met and plans to adjust the process back into control?	7	Minor deficiency

critical limits of CCPs			
6- Establish Procedures for Verification to conform that HACCP system is working effectively.	10. Have verification plans and schedules been developed for each CCP?	3	Major deficiency
	11. Are changes in the process, equipment, ingredients etc., causing timely reviews of HACCP systems, including hazard analysis, CCP decisions, CCP records and staff training?	0	Non-compliance
7- establish documentation concerning all procedures and records appropriate to these principles and their application	12. Is there evidence recorded for HACCP training to all plant employees, including training for CCP operators?	7	Major deficiency

Plant A and Iso: 22000 2005 scored well and has some issues listed in the following:

- CCP records are not being completed properly (Not Enough details in responsibility).
- Single isolated instance in SOP not matching that in manual.
- No evident of review is conducted in order to prevent a recurrence of the situation.
- CCP operator cannot verify their own work.
- Changes to the process, equipment, ingredients, etc., have taken place but there has been no review of HACCP systems.
- Not all plant employees are trained in HACCP.

Jirathana (1998) published a paper by the title Constraints experienced by developing countries in the development and application of HACCP were he found that The problems relate to education and training, the predominant use of the English language, non-uniformity in training manuals, shortage of scientific references and technical information, too few experienced people in industry and in some regulatory authorities, support by top management and the complex nature of some products. The efficiency of auditors, the frequency of auditing, the efficiency of sampling and the way the HACCP systems are developed and implemented.

Sudan being a developing country suffers from most of what was mentioned by (Jirathana, 1998) and it reflects the verification scores of Plant A.

Baş *et al.*, (2007) conducted a similar research in Turkey and the purpose of the study was to determine barriers for HACCP and food safety programs in food businesses in Turkey and they concluded the following:

A lack of understanding of HACCP was identified as one of the main barriers to its implementation 63.5% reported that they did not really know

what HACCP was while 23.5% reported that it was too complicated. Only 33.0% of managers said they had a food safety management system. About 31% of the employees in food businesses had received basic food hygiene training. The majority of managers (91.3%) identified improved customer confidence as a benefit of implementing a food safety management system. Lack of prerequisite programs (92.2%) was the key barrier identified for all food businesses. While lack of knowledge about HACCP (83.5%), lack of time (88.7%), staff turnover (80.9%), lack of employee motivation (83.5%), complicated terminology (87.0%) and lack of personnel training (91.3) was the other most common barriers in food businesses.

Baş *et al.,* (2007) concluded that lack of knowledge about HACCP and other food safety programs were identified as the main barriers for food safety in food businesses. Lack of prerequisite programs and inadequate physical condition of the facility were also identified as other barriers. Training programs, both basic food safety and HACCP to support implementation of prerequisite programs and HACCP in food businesses were suggested.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- Soft drinks from plants A and B were of better microbiological quality that plant C.
- Soft drinks from plant C were the worst in microbiological quality of its products compared to plants A and B.
- All samples from the three plants showed no presence of Coliform bacteria.
- No significant difference between results of microbial integrity of products from Plant a (ISO 22000:2005) and Plant B and (GMP)
- The Three plants nevertheless didn't comply with the Sudanese standard number (250/2007) "Standards of non-alcoholic soft drinks" regarding the microbial limitation but all comply in terms of the freedom from coliform bacteria.
- The results of the three plants fully complied with the gulf standard (Gulf Standard UAE.S/GSO 1016:1998).

5.2 Recommendations

1. To implement quality systems in the soft drinks industry that is to avoid the spread foodborne illness and product spoilage and deterioration due to pathogenic and spoilage microorganisms.
2. The study has found that the establishment of Good Manufacturing Practice program in carbonated soft drinks industry suffices to fully comply with Gulf Standard UAE.S/GSO 1016:1998.
3. Routine audit by relevant authorities shall be present to face cases like Plant C where there was no routine microbiological testing or evaluation is done to their products at the time of study.
4. HACCP training and awareness must be conducted and followed up to insure the proper implementation of HACCP plans.
5. Elaborated study has to be made investigating a wider range of pathogenic and spoilage microorganisms in the final CSDs beverages in the Sudan.

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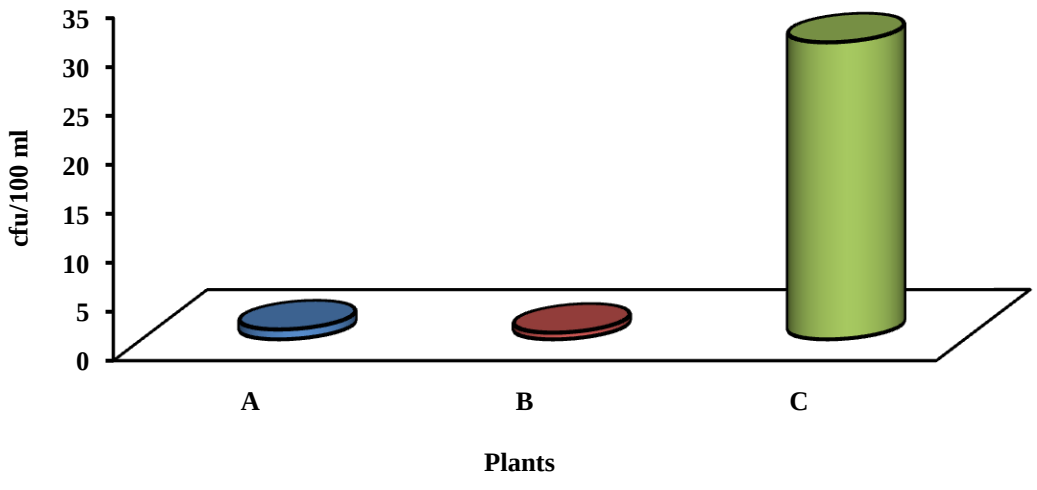
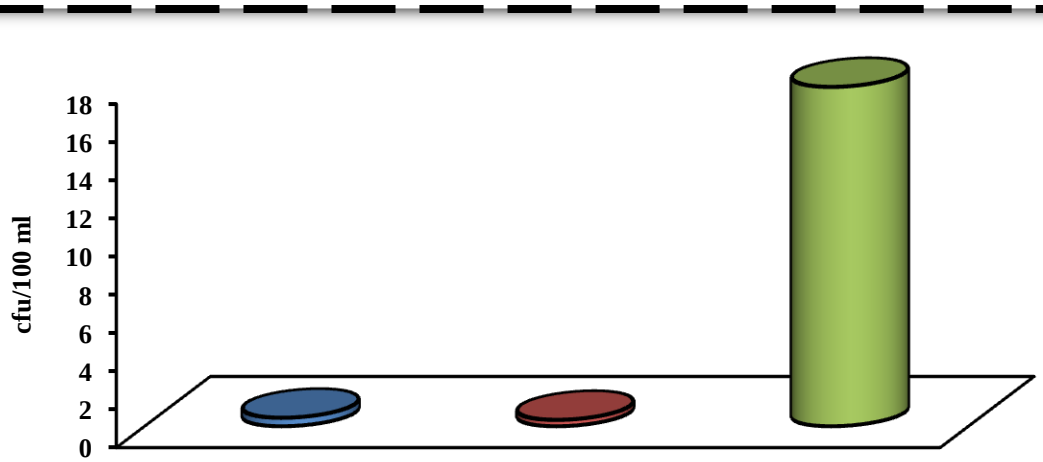
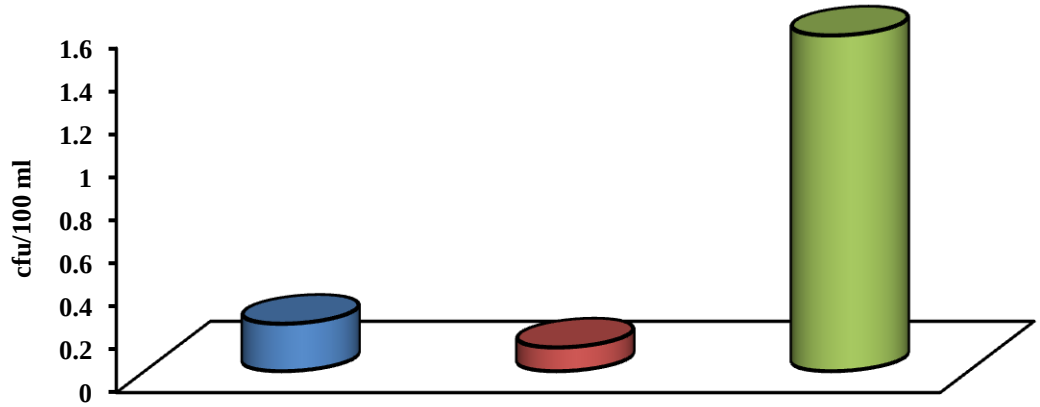
Appendices:



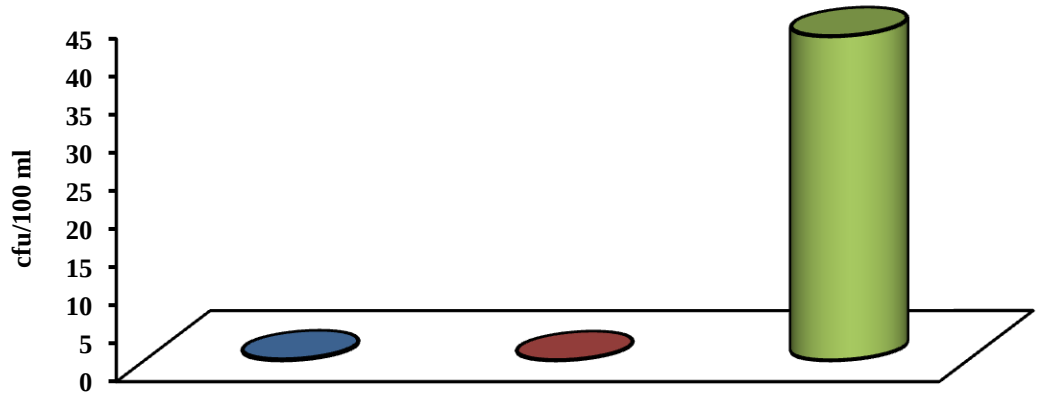
Appendix (1): Total bacterial count in plant A, B and C.



Appendix **(2): Total yeasts** in plant A, B and C.



Appendix **(3): Total mold in plant A, B and C**



Appendix **(4): Total acid tolerant microbes in plant A, B and C**

Appendix 5: Microbiological limits of carbonated beverages.

Item	Microorganism	Limit per ml (as CFU)
Carbonated Beverages	Aerobic Plate count	100
	Coliform	0
	Yeast and Mold	2

- **Source:** (GSO, 1998).

Appendix 6: Microbiological limits of carbonated beverages of the standard SMO (250/2007).

Item	Microorganism	Limit per ml (as CFU)
Carbonated Beverages	Coliform (*E.coli* bacteria, pathogenic bacteria)	0
	Yeast	0
	and Mold	0

- **Source:** (SSMO, 2007).