

**CHARACTERISATION OF A SCUM IN SPORTS DRINK AND DETERMINATION  
OF THE EFFECTS OF PRESERVATION FACTORS ON ITS DEVELOPMENT**

**BY**

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## ABSTRACT

The development of a scum in a commercial sports drink is of concern because the product would be of poor quality, which may result in financial losses due to consumer rejection of the product and hence a decrease in the firm's market share. The scum could be harmful to health and as such the firm could be litigated. Several factors, including microbial proliferation, may be the cause of the development of a scum in sports drink, but the actual cause seems not to have been established. The aim of this study was to characterise the scum in sports drink and determine the effects of preservation factors (pasteurisation, chemical preservatives and refrigeration) on its development.

Samples of the sports drink were taken at different stages of processing to determine the effect of preservatives, pasteurisation and storage temperature on scum development. Some samples were kept at room temperature (approx. 25°C) and others were kept in the refrigerator (approx. 4°C) during the study. A total of 150 samples were analysed over a period of four months. The structural characteristics of the scum that developed in the sports drink were determined by scanning electron microscopy (SEM) and elemental analysis. The sports drink samples were analysed for their microbial load and microbial types. Consumer acceptability of pasteurised and non-pasteurised drink was compared by conducting sensory evaluation using a consumer panel of 60 panellists. Customer complaints recorded by the sports drink manufacture that were due to scum development in the drink were also reviewed to establish the impact of scum development on consumer acceptability of the drink.

The results of the study indicated that scum development was due to microbial contamination of the drink. The causative organism of the scum was identified as *Acinetobacter baumannii*. *Acinetobacter baumannii* is a gram negative non-spore forming coccobacilli and does not ferment sucrose. *Acinetobacter baumannii* forms the scum in sports drink as a means of protection from environmental stresses. The scum was found to be a compound of C, Si and O. The non-pasteurised samples were slightly more acceptable to consumers compared to the

pasteurised samples. The consumer acceptability of pasteurised drink samples was negatively affected by the loss of aroma and flavour during pasteurisation. The preservation factors (chemical preservatives, pasteurisation and refrigeration) had no effect on scum development.

To prevent post pasteurisation contamination, it is recommended that the pasteurisation process be done at the filling stage instead of at the holding stage. The frequency of changing rubbers and gaskets on the filling line should be at least every two months. The drink is pasteurised at 90°C for 20 seconds, this needs to be reduced to a level where it will not have an influence on the loss of taste and aroma of the pasteurised drink, but without reducing the effectiveness of pasteurisation.

## DECLARATION

I, Odwa Mcebisi Mapompo declare that:

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>FAO</b>	Food and Agriculture Organisation
<b>WHO</b>	World Health Organisation
<b>GMP</b>	Good Manufacturing Practices
<b>HACCP</b>	Hazard Analysis and Critical Control Points
<b>CIP</b>	Cleaning In Place
<b>CFU</b>	Colony forming units
<b>VRBA</b>	Violet Red Bile Agar
<b>SEM</b>	Scanning Electron Microscopy
<b>HIV</b>	Human Immunodeficiency Virus
<b>PVC</b>	Poly Vinyl Chloride
<b>EHEC</b>	Enterohemorrhagic <i>Escherichia Coli</i>
<b>EDX</b>	Energy dispersive X-ray analysis

## **ORGANISATION OF THE DISSERTATION**

Chapter 1 presents the Background information, the Research problem, Purpose and Objectives of the study. Chapter 2 presents a Review of the related literature. Materials and Methods are described in chapter 3. Chapter 4 presents the Results and Discussion. The Conclusions and Recommendations are presented after the results and discussion.

## **CONFIDENTIALITY AGREEMENT**

The MSc candidate and the supervisors signed a confidentiality agreement with the firm that produces the sports drink. The agreement is that the results of the study shall be used solely for academic purposes, the results shall not be published, and the name of the firm and the brand name of the drink shall be kept confidential.

## CHAPTER 1

### Introduction and statement of the problem

#### 1.1 Introduction

Quality drinks are clear and free of microbial contamination. Scum in drinks may be caused by various factors, including reaction of the contents of the product or may result from microbial contamination. Microorganisms can be introduced into food through raw materials and processing equipment, but may also come from the environment (Varnam and Sutherland, 1994). With time and in favourable conditions, the microorganisms multiply and lead to spoilage of food (Jos, 1996). In drinks, microbial spoilage may manifest by fermentation, change of sensory properties, and development of a scum. Scum in sports drink is a mucus-like substance that develops in the drink and settles at the bottom of the bottle, floats on top or is sometimes suspended in the drink (personal observation). Scum formation in sports drink is one of the prime indicators of poor quality.

Food spoilage caused by microorganisms is to a large extent preventable using a wide range of preservation techniques, most of which act by inhibiting microbial growth. These include freezing, chilling, drying, curing, conserving, vacuum packing, modified atmosphere packing, acidifying, as well as addition of preservatives (Gould, 1996). Other techniques prohibit the entry of micro organisms to the product e.g. aseptic processing and packaging (Gould, 1996). Besides spoilage by microorganisms, other factors that result in food spoilage are also reported in the literature, these include physical, chemical and enzymatic processes (Gould, 1996).

According to Jos (1996), any change that renders the product unacceptable for human consumption is considered spoilage. Spoilage of food can be physical damage, visible microbial growth, the formation of slime or insect damage (Jos, 1996). The evaluation of spoilage can be related directly or indirectly to sensory assessment, although biochemical or microbial analysis is more objective and more convenient. Although it is difficult to estimate the economic loss that is due to food spoilage, the figures available indicate that it constitutes a high functional loss (Jos, 1996). According to Doyle (2007), it is estimated that more than 96 billion pounds of food were lost in the U.S in 1995 due to spoilage. The development of scum in sports drink has a negative effect on its quality and probably also on consumer safety. The firm could be litigated if a scum in sports drink causes consumer illness. Thus, the

development of scum in sports drink may have a negative impact on the profitability and ultimately on the viability of the firm. This may not only adversely affect the shareholders of the firm, but also the livelihoods, especially food security, of the firm employees and their households. It is therefore of paramount importance that the problem of scum development in sports drink be addressed.

### **1.2 Purpose and objectives of the study**

The main purpose of this study was to characterise the scum developing in sports drink and determine the factors affecting its development so as to find possible ways of preventing it.

#### **Objectives**

- i. To find out whether or not microorganisms are involved in the development of scum in sports drink.
- ii. To identify the causative organism of the scum
- iii. To determine the structural characteristics of the scum developing in sports drink.
- iv. To determine the effect of preservation factors (pasteurisation; chemical preservatives; and refrigeration) on the development of a scum in sports drink
- v. To determine the effect of pasteurisation and scum development on the acceptance of sports drink by consumers.

### **1.3 Study limits**

This study mainly focused on analysing and determining the structural characteristics of the scum developing in sports drink. It was also limited to the analysis of a finished product with main focus on scum development and no attempt was made to alter the product contents or its design.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

This section consists of seven subsections. The first section discusses the sports drink. The second section deals with spoilage microorganisms in soft drinks. The possibility that scum in sports drink is comprised of biofilms is presented in the third section. The fourth section deals with the importance of food quality and safety. The fifth section deals with food preservation. Consumer preference and food quality is reviewed in the sixth section of the study. The last section deals with the possible negative impact of the scum on the firm and its employees.

#### 2.2 Introduction to sports drinks

Shirrefs (2003) defines sports drink as a drink consumed in association with sports or exercise, it can be used prior to exercise, during or after the exercise. Water is the main ingredient in the drink and a variety of nutrients and other substances are dissolved in the water to make up the final product (Shirrefs, 2003). Other key components in sports drink are carbohydrates, electrolytes, such as sodium, potassium and magnesium (Amendola *et al* 2004). The carbohydrates function as an energy source during exercise while the electrolytes serve as fluid replacement s for the electrolytes lost by the body during exercise (Amendola *et al*, 2004). Colourings and flavours are also added to improve the appearance and taste of the product (Shirrefs, 2003). Sports drinks are non-alcoholic and are categorised as soft drinks (Juvonon *et al*, 2011).

#### 2.3 Spoilage microorganisms in soft drinks

Microbial spoilage of soft drinks is mainly caused by bacteria and yeasts. Several types of bacteria are known to spoil foods and beverages. The most common spoilage bacteria in soft drinks are lactic acid and acetic acid bacteria (Juvonon *et al*, 2011). Lactic acid bacteria are a group of gram positive bacteria and can grow under low oxygen, low temperatures and in acidic conditions (Doyle, 2007). According to Juvonon *et al* (2011), under these conditions lactic acid bacteria can cause spoilage of beverages in sealed bottles. Common food spoilage lactic acid bacterial species are *Lactobacillus paracasei* and *Leuconostoc mesenteroids* (Juvonon *et al*, 2011). Other lactic acid bacterial species such as *Lactobacillus brevis*, *Lactobacillus Buchneri*, *Lactobacillus petrolens* and *Weissella confuse* are also found in

contaminated products (Juvonen *et al*, 2011). Some of the lactic acid bacterial strains produce extracellular fructose or glucose polymers from sucrose and cause ropiness of the final product (Juvonen *et al*, 2011). Spoilage of beverages by lactic acid bacteria may also result in fermentation, tainting, slime production and buttery spoilage (Varnam and Sutherland, 1994).

Acetic acid bacteria of genera *Acetobacter* and *Gluconobacter* can cause spoilage of soft drinks (Varnam and Sutherland, 1994). Acetic acid bacteria are gram negative aerobes that exist as motile or non-motile rods (Juvonen *et al*, 2011). High numbers of acetic acid bacteria in production environment is an indication of poor hygiene (Juvonen *et al*, 2011). Acetic acid bacteria can grow in low pH conditions and are commonly detected in soft drinks containing benzoate and sorbate, packed in oxygen permeable plastic containers (Varnam and Sutherland, 1994 and Juvonen *et al*, 2011). Spoilage of beverages by acetic acid bacteria may result in slime and turbidity (Varnam and Sutherland, 1994).

*Bacillus* and *Clostridium* spore formers do not usually grow in soft drinks due to low pH. These bacterial species are common spoilage organisms in less acidic vegetable juices. *Clostridium* species such as *butyricum* and *sporogenes* can cause spoilage in sugar syrups during the production or storage resulting in rancid off-flavour in the finished beverages. These bacterial species can withstand low pH (3.6-3.8) conditions. *Alicyclobacillus* is another spore forming bacterium classified within the genus *Bacillus* (Durak *et al*, 2010). These are gram positive endospore-forming rods. (Juvonen *et al*, 2011).

*Alicyclobacillus* species have been isolated in fruit, fruit juices and beverages. According to Durak *et al* (2010), *Alicyclobacillus* species can withstand low pH (2-4) conditions as well as high processing temperatures (85°C-95°C). Spoilage by *Alicyclobacillus* involves smoky, medicinal, as well as antiseptic off odour derived from guaiacol and halophenols produced by metabolism of the ferulic acid (Durak *et al*, 2010 and Juvonen *et al*, 2011). According to Salo and Wirtanen (2005), yeasts also play a role in the spoilage of certain foods, including juices and other alcoholic and non alcoholic beverages. Yeasts are found in plants, water, animals and insects and thus they are found in various types of foods including industrial raw materials and products (Salo and Wirtanen, 2005). Spoilage yeasts that are commonly found in beverages include *Candida*, *Brettanomyces*, *Saccharomyces*, and *Zygosaccharomyces* (Varnam and Sutherland, 1994).

Yeasts spoilage effects include off flavours, acid and gas formation, swelling of containers and sometimes sediment formation or surface growth (Salo and Wirtanen, 2005). Yeasts are not generally believed to cause food poisoning although a few reports of gastroenteritis or allergenic reactions are said to have resulted from yeast contaminated foods (Salo and Wirtanen, 2005). For a food to be spoiled by yeast, the yeast must be initially present in the food as a contaminant, and depending on the environmental conditions, the yeast may compete and grow quicker than bacteria and moulds (Loureiro and Querol, 1999). The spoilage of food by yeasts can be prevented by selection of good quality raw materials, proper monitoring of processing design to eliminate microbial contamination and by inhibiting or limiting microbial growth (Loureiro and Querol, 1999).

Mould growth is also the cause of spoilage of several types of food (Filterborg *et al*, 1996). Mould growth may result in off flavours, toxins, discolouration, and rot. Moulds produce a number of enzymes that cause food spoilage (Filterborg *et al*, 1996). Enzymatic reactions may result in complete change of the structure of food e.g. change of pasteurised strawberries to strawberry pulp (Filterborg *et al*, 1996). Moulds that are commonly associated with spoiled drinks are of genera *Penicillium*, *Alternaria*, *Aureobasidium*, and *Fusarium* (Varnam and Sutherland, 1994).

Besides microbial growth there are other causes that may result in food spoilage. The appearance of a food, flavour and texture may change due to the action of enzymes. The enzymes may originate from the components of the food or may be produced by microorganisms. The color or texture of a food can also be affected by non enzymatic changes caused by chemicals in the food. The mode of spoilage of a food is predictable and thus, it is important to know the composition of a food in order to assess its likely cause of spoilage (Bell *et al*, 2005).

## **2.4 The possibility that the scum in the sports drink is comprised of microbial biofilms**

According to Kumamoto (2002), a biofilm is a community of microorganisms irreversibly attached to a surface, containing exopolymetric matrix and exhibiting distinctive phenotypic properties. Access of microorganisms in food processing systems can be limited by physical enclosure e.g. sealed processing systems and hygienic practices. In food processing surfaces, biofilms can form in areas that retain moisture and in areas not sufficiently cleaned at regular intervals (Jaykus and Wang, 2009). Poulsen (1999) reported that biofilms are found in cracks, corners, gaskets, joints and crevices in the pipe material and in dead ends in the pipe system. Microorganisms in a biofilm have a broad spectrum of resistance to naturally occurring and intentionally added antimicrobial agents (Bower and Daeschel, 1999). According to Chmielewski and Frank (2003), biofilms in the food industry may contain a high residue of food and mineral content resulting from the product and water used for processing. The residue of food and water content provides protection to microorganisms in a biofilm. Biofilms may be comprised of single or multiple communities of microbial species, they may form a single layer or a three dimensional structure (Chmielewski and Frank, 2003).

According to Xianming and Xinna (2009), many pathogenic microorganisms can form biofilms on food under appropriate conditions. The attachment of these organisms can lead to a problem as they provide a reservoir of contamination. They also increase the risk of contamination in food factories. Food quality and safety can be controlled through application of Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP). Cleaning processes such as cleaning in place (CIP) have also been applied in food processing lines although sometimes even after CIP microorganisms remain and form biofilms on the surfaces of equipment (Xianming and Xinna, 2009). According to Salo and Wirtanen (2005), the cleaning chemicals commonly used in the food industry include chlorine compounds, alcohols, peracetic acids, quaternary ammonium compounds and iodophors. The resistance of biofilms to sanitizers has been tested and it was found that some commercial products including some alkaline detergents and some acid based sanitizers were not effective against some bacterial types of biofilms on stainless steel surfaces (Xianming and Xinna, 2009). It is thus very difficult to completely get rid of microbial biofilms in food processing facilities



No specific information is available in the literature regarding the formation of scum in sports drink. Available information includes reports on tea scum which is mainly due to calcium carbonate ( $\text{CaCO}_3$ ) in water (Spiro *et al*, 1996). Visible mycelium growth has also been reported to occur in spoiled samples of bottled mineral water (Cabral and Pinto, 2001). According to Chmielewski and Frank (2003), improper sanitation of food contact surfaces is a contributing factor in food borne disease outbreaks. Poorly sanitised surfaces will promote soil build up and contribute to the development of bacterial biofilms which may contain pathogens (Chmielewski and Frank, 2003). Jos (1996) reported slime formation in foods as resulting from bacterial contamination. The most common type of food spoilage is microbial spoilage and it may be seen as visible growth (i.e. slime, colonies), textural changes or as off odours and off flavours (Gram *et al*, 2002). Thus it has been assumed that scum in sports drink is comprised of biofilms.

According to Riazi and Matthews (2011), sanitation of food contact surfaces is conducted to inhibit the spread of pathogens and food spoilage bacteria during food processing. Hand sanitizers are also used to inhibit the spread of bacteria from hands and surfaces into food. Sanitizers are highly effective against suspended cells of bacteria than they are effective against bacterial cells in a biofilm (Riazi and Matthews, 2011). Bacteria can develop resistance to sanitizing agents following continuous exposure to sub-lethal concentrations of a particular sanitising agent (Riazi and Matthews, 2011).

Bacterial resistance to sanitising agents also depends on the material in which food is processed, e.g. stainless steel biofilms are much less resistant compared to PVC, and other types of plastic (Pan *et al*, 2006). Glass is sometimes preferred for use in food contact surfaces because its surface can resist corrosion. Stainless steel is much stronger compared to glass, but its surface is less resistant to corrosion, while rubber surfaces deteriorate easily and allow for bacterial accumulation (Bower and Daeschel, 1999). According to Chmielewski and Frank (2003), soil will easily accumulate in corroded surfaces and are more difficult to clean than smooth surfaces. Defects in food contact surfaces provide protection against the removal of microorganisms (Chmielewski and Frank, 2003). Biofilms that form in pipelines can be overcome by using strong alkaline or acid cleaning agents during CIP (Furukawa *et al*, 2010). The resistance of bacterial biofilms to cleaning agents also varies with different types of microorganisms, e.g. *Staphylococcus aureus* biofilms are much more resistant compared to *E. coli* biofilms (Furukawa *et al*, 2010). It is thus important to determine the type of

biofilm in the food processing environment before deciding on a cleaning method (Furukawa *et al*, 2010).

Sometimes the surface microtopography can hinder the effective cleaning when cracks and other surface imperfections protect the bacterial cells from cleaning stresses (Bower and Daeschel, 1999). It is thus also essential that the equipment used for food contact surfaces is carefully designed to avoid accumulation of organic material (Bower and Daeschel, 1999).

It is reported in the literature that bacterial biofilms consist of water, protein, lipid and polysaccharides in addition to the bacterial cells themselves (Jun *et al*, 2010). The ease of release of cells from biofilms in the processing environment is a significant concern in the food industry as pathogenic bacteria may result in contamination of products throughout the different stages of processing. Food processors normally take swabs from food contact surfaces as well as product samples to test for microbial contamination. Results from these swabs and samples may take hours or days to complete and hence the use of rapid techniques such as fluorescence technique is recommended (Jun *et al*, 2010).

According to Jos (1996), food spoilage could also be due to chemical changes. Chemical spoilage in food is sometimes characterised by change in flavour or colour that may result from oxidation, irradiation, lypolysis and heat (Jos, 1996). Chemical changes may take place during processing or storage and they may also result in physical changes like viscosity, gelation and sedimentation (Jos, 1996).

## **2.5 The importance of food quality and safety**

It is critical for a food scientist to understand the nature of the microbial flora likely to grow in a food in order to be able to apply effective ways of maintaining its quality. Information in the literature reveals that microorganisms may grow on food and food contact surfaces (Kumar and Anand, 1998). The popular preservation techniques of pasteurisation and refrigeration are limited in their effectiveness to achieve microbiological quality and safety of foods. While chemical preservatives may be effective at preserving food quality, their addition in excessive amounts may have negative effects on food quality and safety (FAO, 1995). Thus, the chemical preservatives in food must not exceed the dosage levels permitted for normal preservation. This creates a problem for the food industry as certain microorganisms are developing resistance to most preservatives that are used currently in drinks (Brul and Coote, 1999).

Food quality may be affected by physical, chemical, biochemical and microbiological processes (Lee, 2004). Although microbial contaminated foods and beverages may be safe from the public health point of view, they have a limited storage life leading to consumer complaints about the quality of the food. The growth of toxinogenic microorganisms / pathogens in food is the worst form of quality defect as it threatens consumer health (Lee, 2004).

According to the World Health Organisation (WHO, 2007), food safety is an important concern to consumers and governments. Consequently, governments are increasing their efforts to improve food safety. It is difficult to estimate the global incidence of food borne illnesses although it has been reported that in 2005 about 1.8 million people died from diarrhoeal diseases and that was largely attributed to the contamination of food and drinking water (WHO, 2007). A wide spread cholera epidemic resulted in 2700 deaths in the Latin American continent in 1991 (Motarjemi and Kaferstein, 1999). Another food borne disease outbreak, the Enterohemorrhagic *Escherichia Coli* (EHEC) infection broke out in Japan and affected more than 9500 people of which 11 people died as a result of the outbreak (Motarjemi and Kaferstein, 1999). Any country regardless of its development stage can be affected by food borne illnesses and this can result in many deaths, especially children, infants and people with weak immune system (Motarjemi and Kaferstein, 1999). It has been reported that people became ill after consuming unpasteurised juices and wines (Health Link BC File # 72, 2010). These illnesses have been associated with bacteria such as *E.coli* 0157:H7 and 0111 *Salmonella*, *cryptosporidium*, yeast, hepatitis A, norovirus and metal contaminants (Health Link BC File # 72, 2010). Boiling unpasteurised juices before drinking has been reported as the safest way of protecting against the outbreaks of illness at home (Health Link BC File # 72, 2010).

Food and Agriculture Organisation (FAO, 2002) reported that, in developed countries, about one third of the population is affected by food borne illness each year and this is likely to be higher in developing countries. All continents are reporting serious outbreaks of food borne diseases each year illustrating both the public health and social significance of these illnesses (FAO, 2002). The availability of safe food does not only improve the health of the people, but also improves development (FAO, 2002). In addition to the negative effects on people's health and well being, food borne diseases have negative economic effects on individuals, families, communities and businesses. They result in loss of income and perpetuate the cycle of poverty (FAO, 2002). According to the World Health Organisation (WHO), (2010) food

borne illnesses are not only costly to the individuals but also to the society at large. The costs include loss of income, health care costs, loss of productivity owing to being absent from work, loss of sales due to consumers rejecting products associated with food borne illnesses (WHO, 2010). Scum development in sports drink is one of the indicators of poor quality and may have negative implications to the health of the consumers. Therefore, the development of the scum in sports drink may have serious health and economic impacts.

## **2.6 Food preservation**

The principle of food preservation revolves around killing microorganisms or inhibiting their growth. The main aim of food preservation is to minimise the potential for the growth of spoilage and food poisoning microorganisms (Lee, 2004). Chemical preservatives generally preserve food by inhibiting microbial growth. Common chemical preservatives that are used to preserve beverages and sports drinks include, sulphur dioxide, benzoates, sorbates, carbon dioxide, and ascorbic acid (Bates *et al*, 2001). Some of these chemical preservatives only have antimicrobial properties, whilst some of them can retard both microbial and enzymatic activities (Bates *et al*, 2001). Their effectiveness depends on factors such as the pH, composition, acidity, water activity, temperature during processing as well as storage atmosphere of the food (Bates *et al*, 2001; FAO, 1995). In a predictive model developed by Battey *et al* (2001), 225 ppm potassium sorbate was effective in keeping mould growth below 5% at pH 3.2. The same concentration of potassium sorbate was very ineffective at pH 3.8 where mould growth was nearly 90 % (Battey *et al*, 2001). At low pH values titratable acidity, sugar concentration and potassium sorbate were very effective at preventing mould growth (Battey *et al*, 2001). It has been established that low pH environments are barriers to the homeostatic processes of microorganisms (Battey *et al*, 2001). Most preservatives mentioned above will only inhibit microorganisms if they are in the form of undissociated molecules. In this form the preservatives are able to enter the microbial cell, release hydrogen ions, and eliminate the proton gradient across the cell membrane (Harrigan and Park, 1991). Preservatives should be well dispersed throughout the food as this will have an impact on their absorption and diffusion by the cell membranes of microorganisms (FAO, 1995).

According to Brul and Coote (1999), weak organic acids like acetic, benzoic and sorbic acid are also used as preservatives. They act by inhibiting growth of bacterial and fungal cells. Sorbic acid also inhibits germination and growth of bacterial spores. Their mode of action has been proposed to be due to a number of actions including the disruption of cell membranes,

prevention of essential metabolic reactions as well as accumulation of toxic anions (Brul and Coote, 1999). Since these acids are common constituents of food their addition in food as preservatives is not restricted by legislation (Harrigan and Park, 1991). Chelating agents such as citric acid can also act as preservatives since they inhibit microbial growth by chelating divalent metal ions from the food (Nielsen and Arneborg, 2007).

Sodium benzoate and potassium sorbate are the most preferred forms of chemical preservatives because of their greater solubility (Bates *et al*, 2001). In U.S.A benzoates are limited to below 0.1 % and they are mainly used to inhibit the growth of yeast and moulds (Bates *et al*, 2001). Chemical preservatives are added to food in very small quantities and do not change the sensory, physical, and chemical properties of a food (FAO, 1995). Addition of chemical preservatives to foods already contaminated is not recommended since the preservatives may not kill bacteria already present in food (FAO, 1995). The use of preservatives in foods must be limited to those chemical substances that are accepted by national and international standards and legislation (FAO, 1995). There are preservatives that have been unintentionally added to food and these include table salt, sugar and some organic acids (FAO, 1995). Such preservatives are mainly added to improve the quality and sensory properties of a food (FAO, 1995).

Chemical preservatives are not favoured due to the misperception of consumers regarding their safety and the consumers shift towards selecting all natural products (Bates *et al*, 2001). Whilst chemical preservatives are effective in retarding antimicrobial as well as enzymatic and non enzymatic activities, their effectiveness in retarding scum development in sports drink has not been reported in the literature. Harrigan and Park (1991) define pasteurisation as a moderate heating process intended to kill heat-sensitive vegetative cells of microbes in a food with the exception of endospores and other resistant microbes. Vegetative cells die when the temperature is just above the maximum temperature at which the microorganisms grow.

Most microorganisms will die at a temperature of 60°C and above. However there are thermophilic bacteria which are capable of growth at temperatures nearing 100°C. It is possible that pasteurised food may be re-contaminated after the microorganisms have been killed during the pasteurisation process. This is known as post pasteurisation / post heating contamination (Harrigan and Park, 1991). There are different ways in which the post pasteurisation contamination can occur. An example is the post pasteurisation contamination of cans during the cooling stage where the non-sterile water used for cooling is the source of

contamination (Harrigan and Park, 1991). Therefore for the pasteurisation of food to be successful, care must be taken to avoid exposing the food to other sources of microorganisms.

Microorganisms may also exist in a resting state in the form of spores. Spores are much more resistant to heat than the vegetative cells (Harrigan and Park, 1991). According to Eijlander *et al* (2010), control of spores is one of the major problems in food preservation and it is impossible to completely inactivate them without negatively affecting food quality. Spores can survive heat and antimicrobial compounds that kill vegetative cells. Microbial spores are not affected by pasteurisation. Germination of spores is affected by factors such as differences in environmental conditions like temperature, presence or absence of nutrients, chemical compounds in the food and/or the pH of the food (Eijlander *et al*, 2010). According to Bell *et al* (2005), only two bacterial genera are able to produce spores in foods and these are *Bacillus* and *Clostridium* species. *Bacillus* species can grow and produce spores in the presence of oxygen while *Clostridium* species can grow and produce spores in the absence of oxygen (Bell *et al*, 2005). The spore formers of the *Clostridium* species include *Clostridium prefrens* and *Clostridium botulinum* (Nguyen-The, 2012). One of the spore formers of the *Bacillus* species is *Bacillus cereus* (Nguyen-The, 2012).

When faced with unfavourable conditions bacteria capable of producing spores will produce a single spore that will protect their genetic material. The spore is produced inside the vegetative cell which may disintegrate leaving the spore naked. In this state the organism may be inactive but survive until the conditions are favourable for it to grow. The spore will then germinate into a new vegetative cell and repopulate the environment (Bell *et al*, 2005). Most bacterial spores may be killed by heating at 121 °C for about 3 minutes or at 140 °C for 2 to 4 seconds. According to Bell *et al* (2005) heat processes such as pasteurisation could facilitate germination of dormant spores and increase the risk of food poisoning.

Refrigeration extends the storage life of foods by slowing the metabolic processes of microorganisms in food (Russel, 2002). However there are cold-adapted, food poisoning and food spoilage bacteria, such as *Listeria monocytogens* and *Pseudomonas* species that will continue to grow at refrigeration temperatures (Russel, 2002). Therefore the effectiveness of refrigeration will depend on the type of food and the microbial flora growing in the food (Russel, 2002). Inhibiting the growth of microorganisms does not necessarily mean their metabolism will cease. Microbial enzymes may continue to function and spoil food at

refrigeration temperatures (Harrigan and Park, 1991). The preservative effect of refrigeration is achieved by using as low temperature as possible (Harrigan and Park, 1991).

Combined preservation methods commonly referred to as “hurdles”, are also used to preserve food (Bates *et al*, 2001). The principle of using hurdles is based on the thought that while individual methods may sometimes be insufficient to achieve the desired level of preservation, a number of methods combined together may improve product stability (Bates *et al*, 2001). Hurdles can also include modified atmosphere packaging, high hydrostatic pressure, ultraviolet light, ethanol, addition of inhibitory ingredients, altering storage temperature, low pH, etc. (Bower and Daeschel, 1999; Bates *et al*, 2001). Some hurdles (e.g. Maillard reaction products) influence the safety and quality of foods as they have antimicrobial properties and at the same time improve the flavour of the product (Leistner, 2000). Hurdles used for preservation of food are divided into physical, physiochemical, microbially derived and miscellaneous hurdles (Lee, 2004). According to Bell *et al* (2005), the food bacterial spoilage population of preserved food are mostly gram positive. Although they are generally slowly in preserved food gram positive bacteria are more resistant to food preservation than gram negative bacteria. In foods where both gram negative and gram positive bacteria have been eliminated the microbial spoilage tends to be limited to only yeasts and moulds (Bell *et al*, 2025).

Microorganisms can use the homeostasis mechanisms to resist preservation strategies (Leistner, 2000). According to Lee (2004), the function of hurdles is to disturb one or more of the homeostasis mechanisms. This is done by disturbing several homeostasis mechanisms simultaneously, e.g. targeting of cell membranes, DNA, enzyme systems, pH, and water activity (Lee, 2004). Employing hurdles / combined methods of smaller intensity is much more effective than using one preservative of a larger intensity (Leistner, 2000). Hindering of the microbial growth may also include rotation of antimicrobial agents and sanitizers used for disinfection of food contact surfaces (Bower and Daeschel, 1999).

It is important to note that good sanitation practices are the first barriers in inhibiting microbial growth (Bates *et al*, 2001; Bower and Daeschel, 1999). Cleanliness of raw materials and production surfaces including personnel is very important in quality of processed or preserved food. This is because most preservation techniques act by preventing growth rather than killing or inhibiting the microbial growth (Harrigan and Park, 1991). Food preservation, either using a single or a combination of preservation hurdles can have a

negative effect on the sensory properties of food. For example, over-pasteurisation can negatively affect the flavour, smell and appearance of the product (He *et al*, 2005); the pH of fermented sausages is lowered to prevent microbial growth but the pH may become very low and impair the taste of the product (Leistner, 2000). To achieve a product that meets the desired sensory properties while also stable and safe, it is important to use combined preservation methods because each preservation hurdle is applied at a relatively lower intensity than that would be applied if the hurdles were used individually (Lee, 2004).

## **2.7 Consumer preference and food quality**

According to Clark (1998), food is not just consumed to obtain nutrition and a healthy happy body. The other aspect of food consumption is that consumers want to enjoy their food (Clark, 1998). There are some foods that are simply consumed for the pleasure value they impact. Such products include chocolate, coffee, ice cream, and beverages (Clark, 1998). There is some evidence that the sensory characteristics (i.e. taste and flavour) of food have some effect on the consumer's food choice (Clark, 1998). When consumers experience the sensory characteristics of food they are able to make the choice whether they like the food or not. Optimising the perceived sensory properties of a product can help increase its consumer perceived value (Clark, 1998).

It is the goal of every manufacturer to optimise their products in terms of consumer preference (Murray and Delahunty, 2000). Companies that produce products that satisfy consumer requirements are successful and profitable (Murray and Delahunty, 2000). The sensory appeal of a food has a powerful influence on consumer acceptability of products (Murray and Delahunty, 2000). If the consumer expectations are not met by the sensory delivery of a food, consumer disconfirmation may result (Murray and Delahunty, 2000). Consumer acceptance and preference of food is generally affected mainly by its taste, texture, colour (or appearance) and aroma (Birch *et al*, 1977).

It is suspected that scum development in sports drink may negatively affect the sensory attributes of the product. This may result in the product being less accepted and/or preferred by the customers and thereby result in loss of market share. Lore *et al* (2004) reported that the rejection of milk by consumers in sub-Saharan Africa and the Near East was due to the spoilage and adulteration of the product. Consumers expect to buy food that is wholesome, unadulterated, nutritious, safe and of standard quality (He *et al*, 2005). The quality of food



indicates its fitness for use, and that it meets biochemical, sensory and physical requirements (Wangalachi, 2003). According to Wangalachi (2003), the presentation of poor quality and unsafe food may not only have undesirable consequences to the consumers but may also affect producers and marketers. Such consequences may include spoilage of the food, disease outbreaks, loss in expected revenue due to rejection, and product recalls.

Production of poor quality food also places the firm in a difficult situation of recovering product credibility and customer good will (Wangalachi, 2003). It is also very important for the food producers to understand why consumers prefer some foods to others for the purposes of marketing and product development (Clark, 1998).

### **2.8 Scum in sports drink may impact negatively on the firm and its employees**

In a survey conducted in Palestine by the Food and Agriculture Organization (FAO) in 2004, it was found that the rate of unemployment and job losses were amongst the economic reasons that resulted in food insecurity (FAO, 2005). After the survey, FAO recommended that in order to improve the economic access to food it was significant to launch a substantial employment creation programme (FAO, 2004). According to Hishamunda and Ridler (2006), the two main components of food security are access and availability of food. Food accessibility is mainly favoured by generation of employment. The statistics presented by FAO in 2003 indicated that the number of people who are food insecure in the world is about 800 million people. In some regions like sub-Saharan Africa the number of food insecure people is expected to rise by 27% by the year 2012 (Hishamunda and Ridler 2006). Food insecurity is partly due to the absence of economic growth and the fact that most people are unable to access food due to lack of income (Hishamunda and Rilder, 2006).

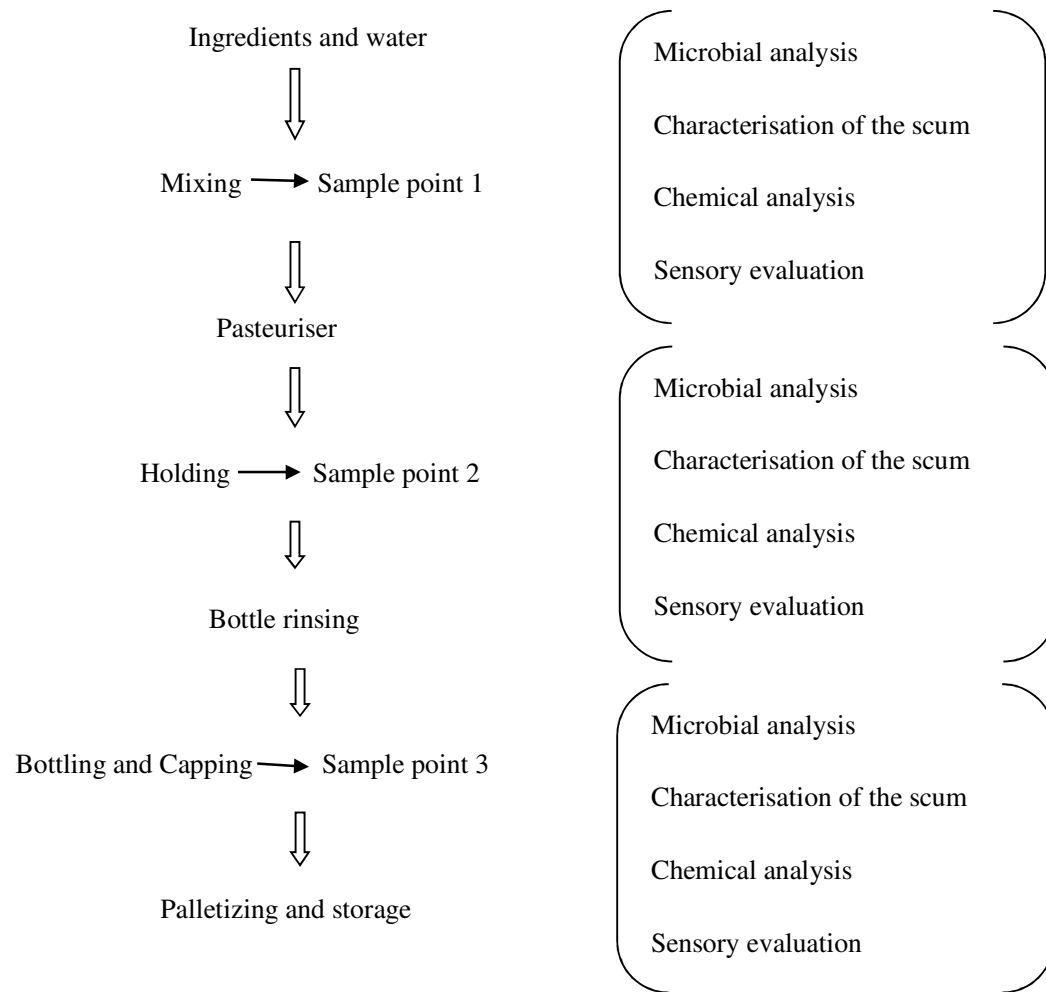
Like any other manufacturing sector, the production of sports drink is vital in food security as it contributes to job creation and generation of income for the poor. The quality of this drink needs attention both for food safety purposes and creation of job opportunities.

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1 Sports drink processing**

The main operations in the processing of sports drink are mixing of ingredients, pasteurisation, bottling and capping. Stages which were thought to be critical points for microbial growth were chosen as sample collection points (Figure 1). The product was processed in a juice processing plant during the normal production hours. The surfaces of the processing equipment were cleaned following the cleaning in place (CIP) process. Water was added to the mixing tank to the desired level. The ingredients were then added in desired amounts. The product was mixed and pasteurised at 90°C for 20 seconds. After pasteurisation the drink was transferred into the holding tank. The product was then drawn from the holding tank into an automatic filling line. Sample points were cleaned and flame sterilized using 95% ethanol before sampling. Samples were then carefully drawn and collected into sterile PET bottles. The bottles were capped, marked and taken to the laboratory for storage.



**Figure 1:** Flow chart showing different stages of processing where samples were taken for analysis

### 3.2 Experimental design

The experimental design applied was such that 60 samples of the sports drink were taken from Sample point 1 (Figure 1). At this sample point 30 samples were taken before the preservatives were added and the other 30 samples taken after the addition of preservatives. The samples were analysed for the presence of scum. Scum developing in the sports drink was examined for its structural characteristics. This was done to address the third objective of the study. Samples from Sample point 1 were tested to partially address the fourth objective. The other 30 samples were taken after pasteurisation also to address part of the third objective. A further 60 samples were taken after bottling and capping to verify if there was any post pasteurisation microbial contamination, which would lead to the development of scum. From the last 60 samples, 30 samples were kept at a controlled temperature (4°C) and the other 30 were left at room temperature (approx. 25°C) and this also relates to the fourth objective. Sensory evaluation was conducted to address the fifth objective of the study.

### 3.3 Sample analysis

#### 3.3.1 Microbiological analysis

The microbial load of the samples was determined using the spread plate method (Bell *et al*, 2005). Serial dilutions were done only up to  $10^5$  dilution. The results were expressed in colony forming units (cfu /ml). Tests that were done included total plate count (TPC), total coliform count, yeasts and mould count, spore formers and bacterial identification. Sterile agar (TPC, VRB, Chloramphenicol, and nutrient agar) was poured into empty petri dishes and allowed to set. Exactly 0.1 ml of each sample was then aseptically transferred into the labelled petri dishes. The samples were then carefully spread evenly over the agar surface using a sterile spreader.

The medium used for the TPC was the total plate count agar. The total plate count agar plates were incubated at 35°C for a period of 48 hours and counts were carried out and recorded (Harrigan, 1998). For yeasts and moulds, the chloramphenicol agar was used. The plates were incubated at 25°C for 5 days after which the colony forming units were counted (Harrigan, 1998). Violet red bile agar (VRBA) was used for the total coliform count. The agar plates used for the analysis were incubated at 35°C for 24 hours (Harrigan, 1998). For the determination of the spore formers, nutrient agar plates were used. Samples were heated in a water bath set at 80°C for 15 minutes to kill vegetative cells (Bell *et al*, 2005). The plates

were enclosed in large plastic bags to minimise dehydration of the media (Harrigan, 1998). The plates were then incubated at 55°C for a period of 3 days after which colonies were counted. All colonies that grew during incubation were counted.

The colonies were counted using the formula:  $\text{count/ml} = \text{number of colonies counted} \times \text{reciprocal of dilution} \times \text{reciprocal of dilution volume plated}$  (Bell *et al*, 2005). The morphology of the bacteria was then determined using Gram stain technique as explained by Harrigan (1998). Gram reactions and shapes of the cells were then recorded.

Bacterial identification was carried out by Microchem laboratory in Gauteng (Morehill, Benoni, South Africa).

### **3.3.2 Determination of the presence and characterisation of scum**

The presence of scum was determined weekly by visual observation. Samples containing the scum were isolated. An attempt was made to determine the structural characteristics of the scum by light and scanning electron microscope. The scum was separated out of the drinks and placed into 0.5 µm filter membrane. The membranes were adhered to sample stubs using a two way tape and allowed to air dry. Samples were then coated with gold and viewed under a scanning electron microscope (ZEISS VOELS15) at 8 KV. The instrument is also equipped with energy dispersive X-ray analysis (EDX) detector which was also used to conduct elemental analysis to determine the chemical composition of the scum in this study. Samples were prepared following the same procedure used for the scanning electron microscope. The principle of elemental analysis using a scanning electron microscope equipped with an EDX detector is explained by Voutou and Stefanaki (2008). The scanning electron microscope electron probe scans the sample and excited X-rays are emitted from atoms of the chemical elements making up the sample. The EDX detector captures the emitted X-rays. The energy of the X-rays is characteristic of the element from which the X-rays were released.

### **3.3.3 Physico-chemical analysis**

The total dissolved solids (TDS), pH and titratable acidity of the sports drink were measured. TDS was measured with a refractometer. Titratable acidity was determined by titration of the sports drink samples against 0.1 M NaOH. Phenolphthalein was used as the indicator of the end point. Exactly 10 ml of the sample was transferred into an Erlenmeyer flask. Exactly 60 ml of distilled water was added into the sample. Three drops of the indicator was added. The

solution was titrated with the NaOH solution until the colour of the solution turned to faint pink. Percentage citric acid in the solution was calculated as titre X 0.64 / 10.

### **3.3.4 Sensory evaluation**

The purpose of the sensory evaluation was to determine the effects of pasteurisation and scum development on consumer acceptability of the sports drink. This evaluation was done to address the fifth objective of the study. Ethical approval to conduct sensory evaluation was obtained from the University of KwaZulu-Natal (UKZN) Research Office. The sensory evaluation included determination of taste, smell, appearance and overall acceptability. The most important sensory attributes that were measured are the taste and smell of the drink. Samples used for the evaluation were free of scum development. This was done for consumer safety.

The evaluation was done using the 9 point hedonic scale described by Nicolas *et al* (2010). The 9 point hedonic scale is comprised of a series of nine verbal categories ranging from dislike to extremely dislike (Nicolas *et al*, 2010). For quantitative and statistical analysis the verbal categories were converted to numerical values, “like extremely” as 9, “dislike extremely” as 1 (Nicolas *et al*, 2010). A scoring guide for the evaluation was given to the consumer panel at the time of evaluation. The panellists were given a short training on how to score the samples on the evaluation form.

Sixty UKZN students of different ages and gender evaluated the samples sensorially. The distribution of the consumer panel was 38% males and 62% females (Table 2). The questionnaire was given to each panellist together with the samples. The samples were marked with 3-digit codes generated from a Table of Random Numbers to reduce bias. The serving order of the samples was randomised using a Table of Permutations of Nine. Water was provided to the panellists to rinse their mouths in between the tasting of samples. The evaluation was conducted two weeks after sample production of the sports drink to establish if pasteurisation of the product had any influence on its acceptability to consumers. The temperature of the serving room was ambient (about 25°C). In addition to sensory evaluation conducted at UKZN, customer complaints recorded by the firm during the period of this study were also assessed to determine the impact of the scum on consumer acceptability of the sports drink

## CHAPTER 4

### 4.1 RESULTS AND DISCUSSION

#### 4.1.1 Microbiological changes

The microbial analysis of the samples from week one to week five after production showed no bacterial growth. The samples were then kept and observed for the development of the scum. The scum was visible after week 10 and the microbial analysis was resumed from week 11. This was done to establish if there was any role played by microorganisms in the development of the scum. There was no scum development on samples taken before and after pasteurisation but the scum developed only on some of the samples collected at the filling stage. Samples with no scum development had zero microbial growth. The scum appeared on samples stored at room temperature and also on refrigerated samples. All samples with the scum had chemical preservatives added. This shows that the preservation factors used were unable to prevent scum development in the drink. Total plate count ranged from  $1 \times 10^4$  to  $>3 \times 10^5$  cfu/ml. There were no yeasts, coliforms or spore formers detected. It was suspected that the scum was due to post packaging contamination, the source of the microorganisms could have been from dirty bottles, but this is subject to further investigation as it was not part of this study. The results are shown on Table 1.

**Table 1:** Microbiological population results of the drink containing the scum

Sample number	Total plate count	Coliforms	Yeasts & moulds	Spore formers	Cell morphology	Gram reaction
1	1.5x10 <sup>5</sup>	ND	ND	ND	Coccobacillus	-
2	2x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
3	5x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
4	2x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
5	3x10 <sup>5</sup>	ND	ND	ND	Coccobacillus	-
6	8x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
7	3x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
8	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
9	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
10	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
11	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
12	2x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
13	2x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
14	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
15	1x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
16	2x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-
17	1.6x10 <sup>5</sup>	ND	ND	ND	Coccobacillus	-
18	1.5x10 <sup>5</sup>	ND	ND	ND	Coccobacillus	-
19	1.3x10 <sup>5</sup>	ND	ND	ND	Coccobacillus	-
20	6x10 <sup>4</sup>	ND	ND	ND	Coccobacillus	-

ND = not detected

Samples 1-10 were stored at room temperatures (approx. 25°C)

Samples 11-20 were stored at a cold temperature (approx. 4°C)

All samples had chemical preservatives added

All samples were pasteurised

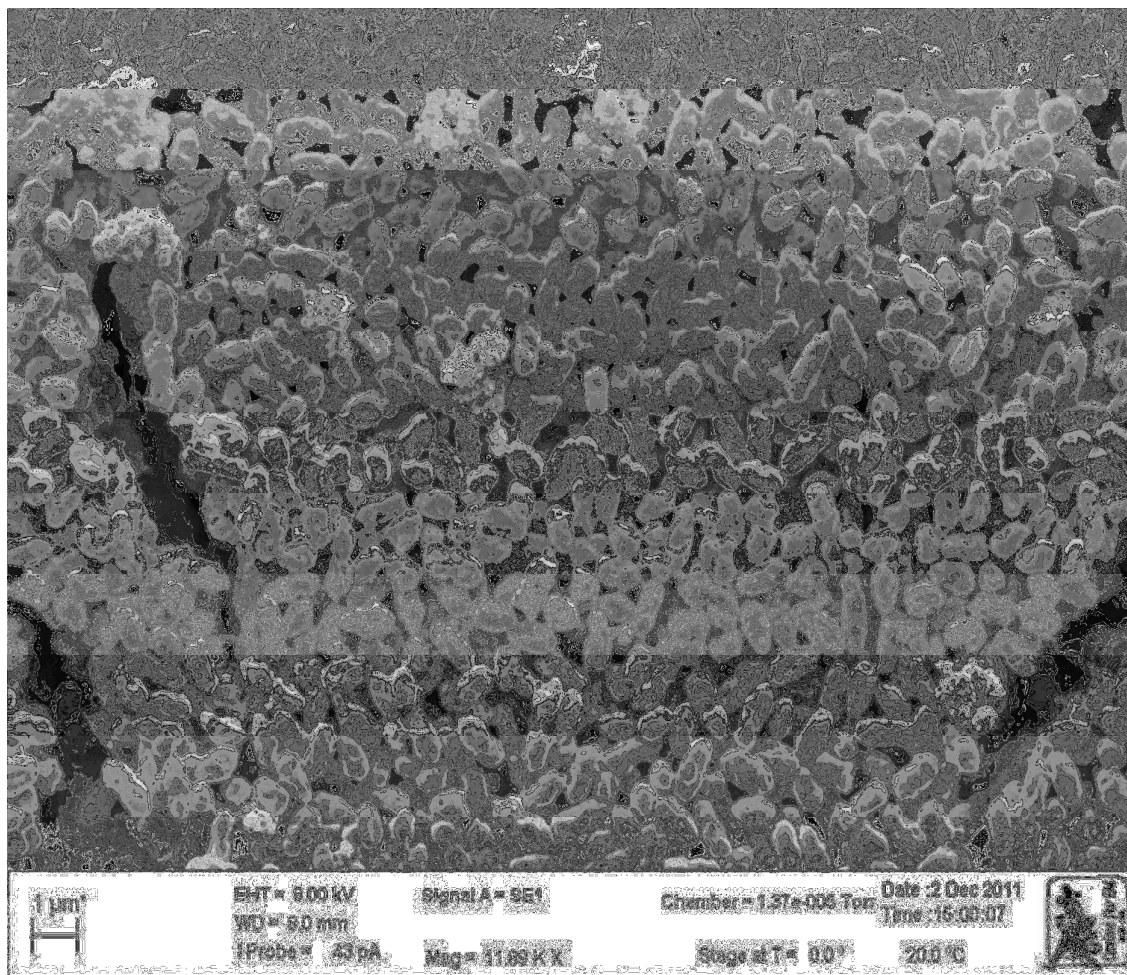


#### 4.1.2 Bacterial identities

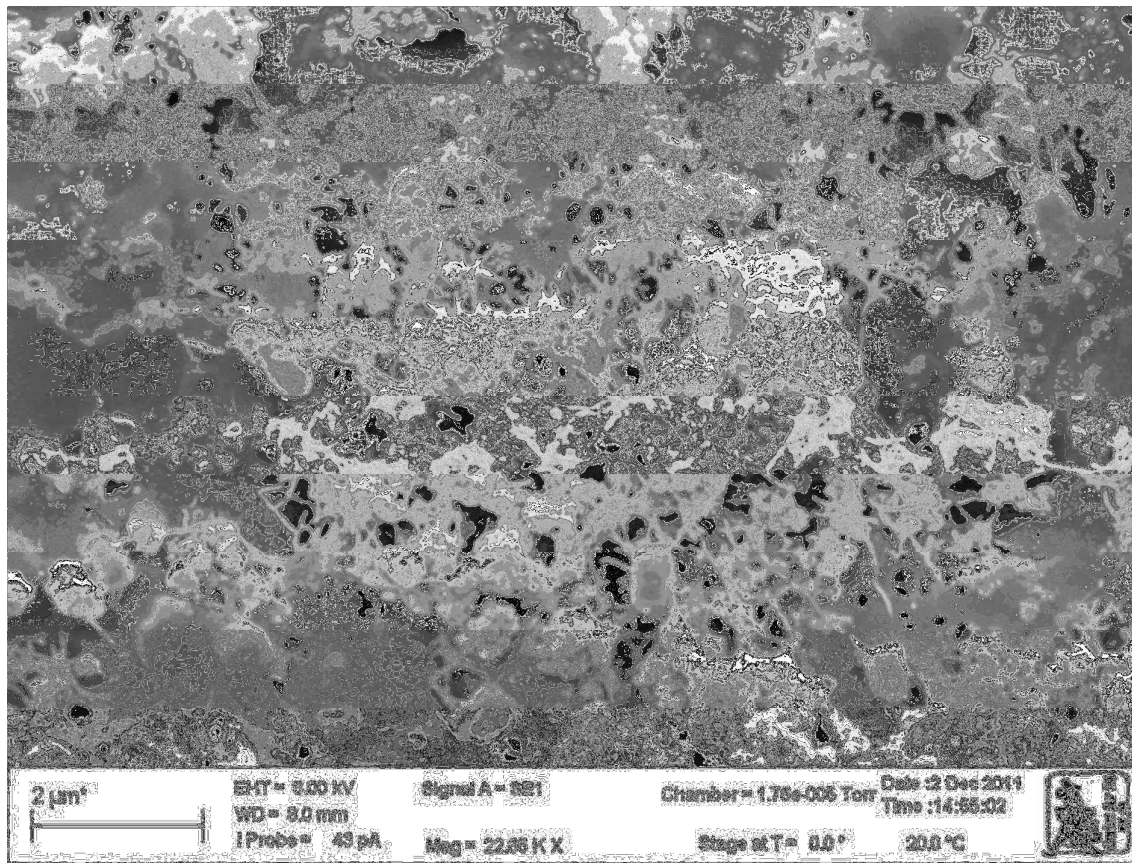
*Acinetobacter baumannii* was identified as the probable causative organism of the scum. No yeasts or coliforms were identified; spore formers were also not identified. In the literature, *A. baumannii* is documented as pathogen that can cause infections such as pneumonia, urinary tract infections, bacteraemia and meningitis (Espinal *et al*, 2012). This bacterium is found in soil and in water but has also been isolated in human skins and in food products (Giamarellou *et al*, 2008). According to Patwardhan *et al*, (2008) members of the *Acinetobacter* genus are gram negative, non-motile, non-spore forming coccobacilli. *A. baumannii* is capable of producing acid from glucose, xylose, galactose, mannose, rhamnose and lactose but is not capable of producing acid from mannitol and sucrose (Constantiniu *et al*, 2004). According to Dheepa *et al* (2011), *A. baumannii* is able to resist physical and chemical disinfection through the formation of a biofilm. Shih and Lin (2010), described *A. baumannii* as one of the water pathogens commonly found in chlorinated potable water, which can exist in both free flowing planktonic cells as well as in biofilms adhering to the inner surfaces of pipes. *A. Baumannii* can survive on finger tips, glass, plastic and other environmental surfaces (Espinal *et al*, 2012). The findings suggesting the occurrence of *A. baumannii* in the sports drink is of great concern because the microorganism is capable of biofilms and is pathogenic which would impact negatively on the quality and safety of the drink. There is a need to increase the intensity of sanitary and preservation factors post pasteurisation in order to prevent the occurrence and proliferation of the suspected bacterium and other microorganisms that may occur alongside it.

#### 4.1.3 Characteristics of the scum as viewed by scanning electron microscopy (SEM)

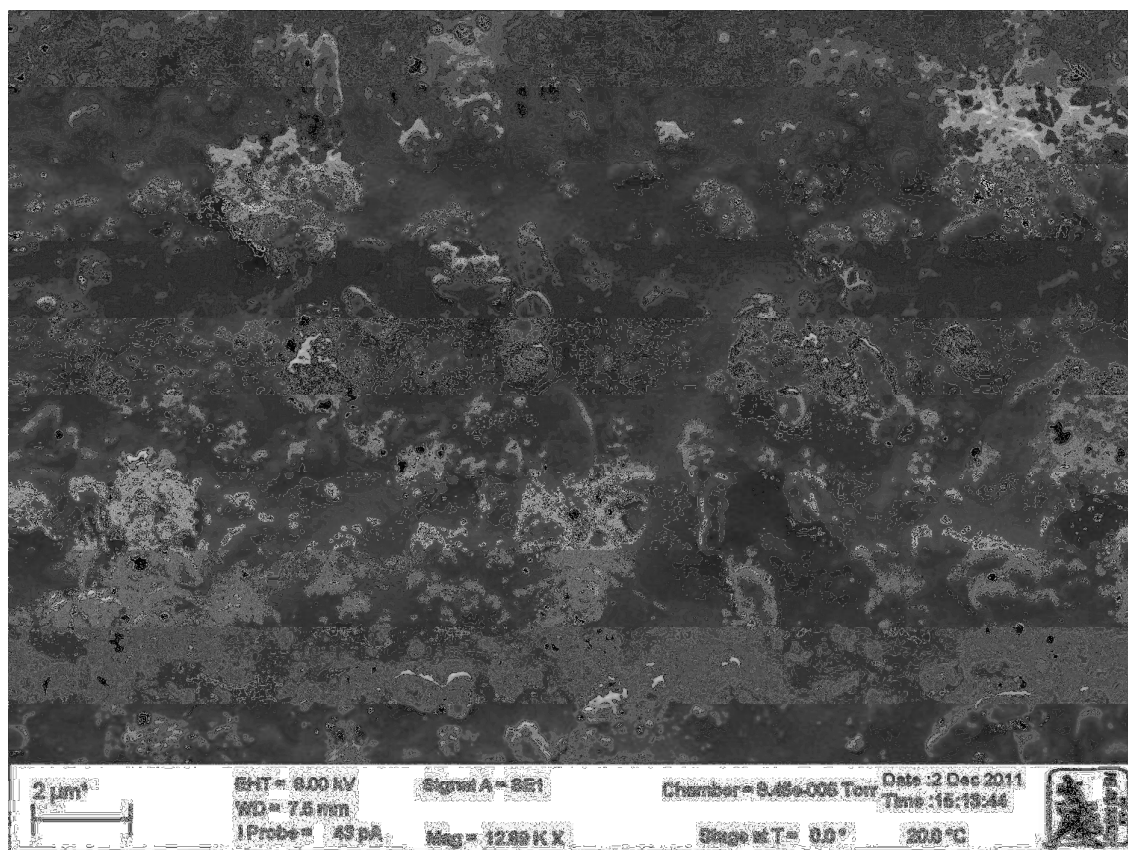
SEM micrographs of the scum are shown in Figures 2-4. The micrographs indicate that the scum consisted of coccobacilli bacterial cells (Figure 2). This corresponds with *A. baumannii* characteristics described by Patwardhan *et al* (2008). The micrographs showed that the fully matured scum had portions of clustered white granular matter (Figure 3). Figure 4 shows the scum surrounding the bacterial cells. Elemental analysis showed that the white granular matter was silica. The scum was characterised to be a compound of carbon, silica and oxygen. The elemental analysis pictures (Figures 5-6) show the difference between a developing scum and a fully developed scum. Fully developed scum had more silica (4.49%) compared to the developing scum which had 1.66% silica. The scum is therefore a layer of biofilm / protective substance produced by bacterial cells.



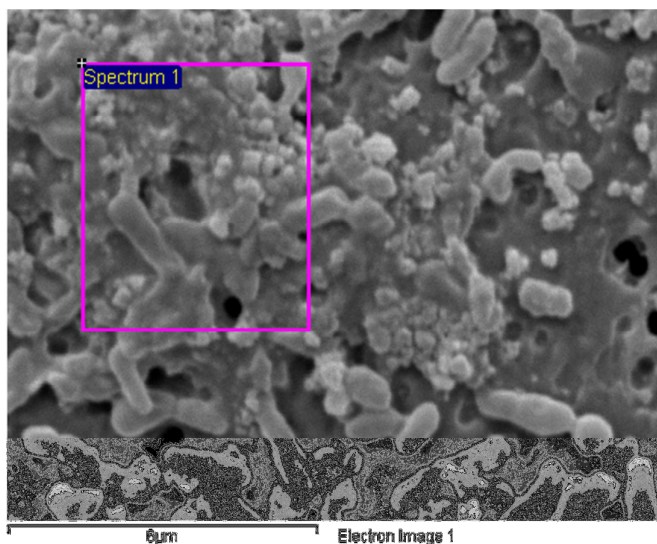
**Figure 2:** SEM micrographs showing coccobacilli shaped bacterial cells in the scum of the drink



**Figure 3:** Fully matured scum with portions of white clustered matter determined as silica



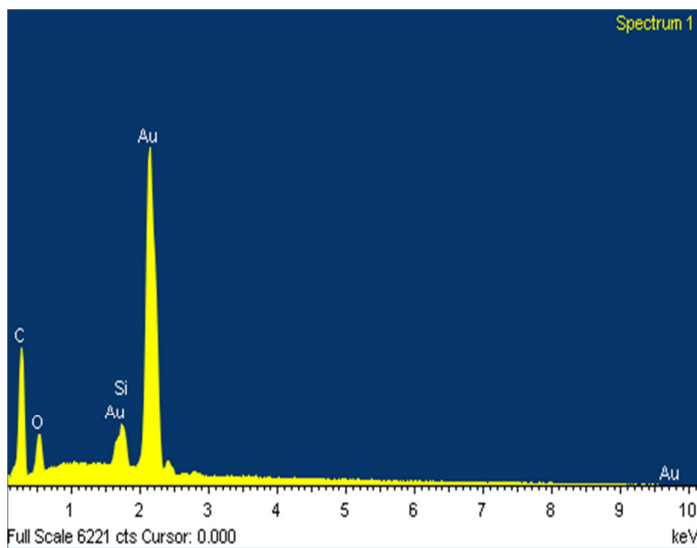
**Figure 4:** The scum on the bacterial cells



(a) SEM micrograph showing elemental analysis on a developing scum

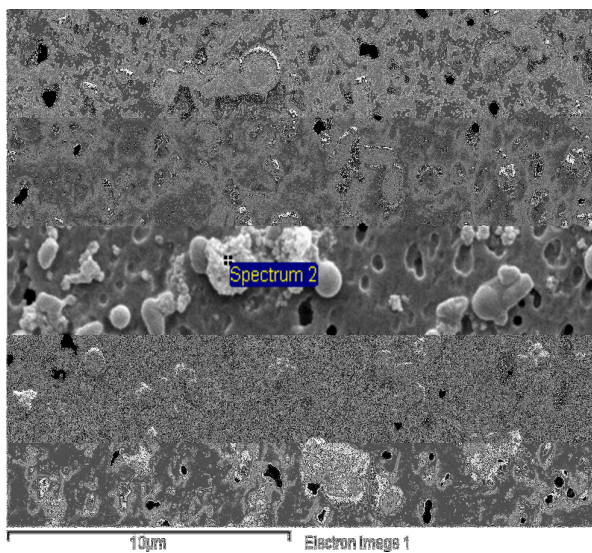
Element	Weight %
C K	18.44
O K	4.38
Si K	1.66
Au M	75.52
Totals	100

(b) Chemical composition of a developing scum



(c) EDX spectrum showing elements of a developing scum

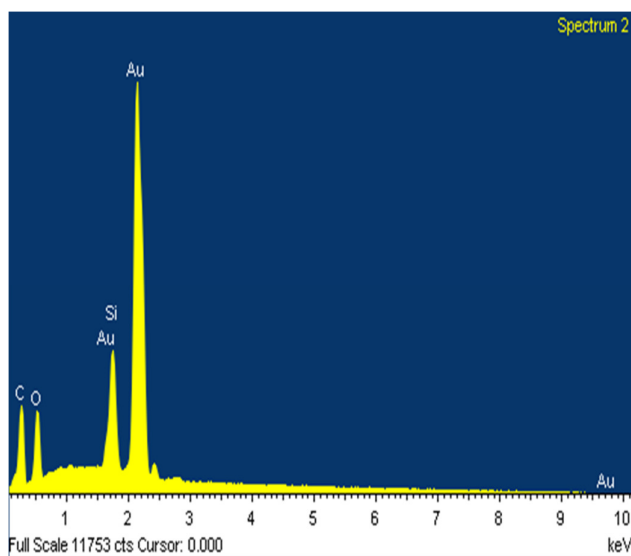
**Figure 5:** Elemental analysis on a developing scum in sports drink



(a) SEM micrograph showing elemental analysis on a fully developed scum with white matter

Element	Weight %
C K	10.33
O K	6.84
Si K	4.49
Au M	78.33
Totals	100

(b) Chemical composition of a fully developed scum



(c) EDX spectrum showing elements of a fully

**Figure 6:** Elemental analysis on a fully developed scum in sports drink

#### **4.1.4 Physico-chemical quality of the sports drink**

The results of physico-chemical analysis of the samples are shown in appendix C. The physico-chemical analysis of the samples indicated that there were no differences in pH and titratable acidity of the normal samples and samples containing the scum. The standard error between the samples showed no significant difference (Appendix C). The TDS of the samples also showed no significant difference. No gas or acid production was evident in the samples. The physico-chemical analysis results also correspond with the *A. baumannii* characteristics described by Patwardhan *et al* (2008), since the bacterium did not produce acid from sucrose in the drink.

#### **4.1.5 Effect of pasteurisation on consumer acceptability of the sports drink**

The first sensory evaluation of the sports drink was conducted two weeks after the samples were collected from the production line. This was done to compare consumer acceptance of pasteurised and non pasteurised samples. The profile of the consumer panel is shown in Table 2. Results of sensory evaluation are shown in Table 3 and analysis of variance (ANOVA) of the sensory evaluation results is presented in appendix B.



**Table 2:** Gender participation in sensory evaluation of sports drink

Gender	Frequency	Percent
Male	23	38.3
Female	37	61.7
Total	60	100.0

**Table 3:** Sensory evaluation of sports drink

Sample	Taste	Smell	Appearance	Overall acceptability
Un-pasteurised	6.8 <sup>a</sup> ± 1.6	6.7 <sup>a</sup> ± 1.5	6.6 <sup>a</sup> ± 1.7	6.9 <sup>a</sup> ± 1.4
Pasteurised	5.9 <sup>b</sup> ± 1.9	6.4 <sup>b</sup> ± 1.5	6.6 <sup>a</sup> ± 1.7	6.3 <sup>b</sup> ± 1.8

Mean ± SD. N = 60. Means with different superscript letters are significantly different ( $p < 0.05$ )

Table 3 shows that the taste of non-pasteurised sports drink samples was slightly more acceptable (score: 6.8) to the panellists compared to the pasteurised drink (score: 5.9). Likewise the smell of the unpasteurised sports drinks samples was more acceptable (score: 6.7) compared to the pasteurised (score: 6.4) samples. This was likely due to the loss of the typical non-cooked drink flavour and aroma in pasteurised samples as was stated by He *et al*, (2005). The pasteurised and the unpasteurised drinks were equally acceptable in terms of appearance (score: 6.6). The overall acceptability of the pasteurised drinks was slightly affected by loss of taste and smell ( $p = 0.05$ ) with unpasteurised drinks (score: 6.9) being more acceptable than the pasteurised drinks samples (score: 6.3). Appendix B (ANOVA) shows the difference in consumer acceptability of the pasteurised and non-pasteurised drinks. The taste of pasteurised and non-pasteurised drinks was significantly different.



The samples were then left at room temperature to observe the development of the scum. No scum development was observed on samples taken before and after pasteurisation. Scum development was observed on pasteurised samples which were collected down the line after filling. The scum did not develop on all samples but only a few. It was then concluded that the scum resulted from post pasteurisation contamination during the filling of the sports drink into the bottles. The scum development can take from two to ten weeks to develop depending on the level of contamination and the conditions in which the product is stored after packaging (personal observation). Looking at the customer complaints report recorded by the firm it was evident that the development of the scum did have an effect on the acceptability of the sports drinks to the consumers. Customers simply rejected sports drinks containing a scum. This also resulted in some of the batches of the finished product being recalled from the market. Recalling of a batch including the cost of dumping was estimated to be R30 000-35 000. If the development of the scum in the sports drinks could be prevented this loss of revenue could be avoided and the company could realise an increase in its market share.

#### **4.1.6 CONCLUSIONS AND RECOMENDATIONS**

It was concluded that the scum was due to contamination of the sports drink with the bacterium *Acinetobacter baumannii*. The findings of this study indicate that the scum is a compound of C, Si and O. It is probable that the scum was produced by the bacterium identified and has a protective function. The scum developed across all samples, those stored at room temperature and in refrigerated storage. It is thus highly recommended that strict sanitation practices, such as the changing of gaskets every two months be followed to ensure the cleanliness of production surfaces and to prevent the build-up of biofilms on the production line surfaces. The pasteurisation of the drink should be done at the filling stage instead of pasteurising in a holding tank. Once the samples have been contaminated neither addition of preservatives nor refrigeration will prevent scum development. Sports drink samples that were taken immediately after pasteurisation of the product did not have any scum and this supported the post pasteurisation contamination hypothesis.

Customer complaints indicated that the sports drink containing the scum was unacceptable. On the other hand sensory evaluation of the sports drink conducted in this study indicated that the pasteurised drink was significantly less acceptable to consumers when compared with the non-pasteurised drink. The acceptability of the sports drink could be increased by

reducing the intensity of pasteurisation, applying strict hygiene post pasteurisation and increasing the concentration of chemical preservatives, but not to the detriment of consumer acceptance of the drink.

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## Appendix A: Sensory evaluation instructions and sensory evaluation form

### Sensory evaluation instructions:

- Please rinse your mouth with water before starting. You are also required to rinse your mouth with water after tasting each sample.
- Please do not smoke 30 minutes before or during the evaluation.
- Please do not consume any food products 15 minutes before the evaluation.
- Please do not communicate with other panellists during or after the evaluation.
- Please rate the taste, smell, appearance and overall acceptability of the samples according to the scale given below. Please taste samples from left to right

### 9-point hedonic scale guide

9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

## Sensory evaluation form

Sports drink evaluation form					
Evaluator no.....					
Sample code	Taste	Smell	Appearance	Overall acceptability	Comments
973					
108					
Additional comments:					

## Appendix B: Sensory evaluation ANOVA

ANOVA (analysis of variance) of sensory evaluation results

		Sum of Squares	df	Mean Square	F	Sig.
Taste	Between Groups	22.533	1	22.533	7.029	.009
	Within Groups	378.267	118	3.206		
	Total	400.800	119			
Smell	Between Groups	1.633	1	1.633	.746	.389
	Within Groups	258.233	118	2.188		
	Total	259.867	119			
Appearance	Between Groups	.000	1	.000	.000	1.000
	Within Groups	350.800	118	2.973		
	Total	350.800	119			
Overall acceptability	Between Groups	10.208	1	10.208	3.932	.050
	Within Groups	306.383	118	2.596		
	Total	316.592	119			

### Appendix C: Physico chemical quality

Total dissolved solids before and after scum development

Before scum development	After scum development	Standard deviation	Standard error
7.2	7.21	0.01	0.007
7	7	0	0
7.1	7.09	0.007071068	0.005
7.2	7.21	0.007071068	0.005
7.15	7.16	0.007071068	0.005

pH of samples before and after scum development

Before scum development	After scum development	Standard deviation	Standard error
3.2	3.19	0.00707107	0.005
3.1	3.09	0.00707107	0.005
3.3	3.31	0.01	0.007
3	2.95	0.03535534	0.025
3.2	3.17	0.0212132	0.014

Titrateable acidity of samples before and after scum development

Before scum development	After scum development	Standard deviation	Standard error
0.21	0.2	0.007071	0.005
0.22	0.25	0.021213	0.015
0.2	0.18	0.014142	0.009
0.25	0.24	0.007071	0.005
0.2	0.19	0.007071	0.005