AIRBORNE MICROBIOTA AND RELATED ENVIRONMENTAL PARAMETERS ASSOCIATED WITH A TYPICAL DAIRY FARM PLANT

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DECLARATION OF INDEPENDENT WORK

I, the undersigned, do hereby declare that this research project submitted to the **Central University of Technology, Free State**, for the degree **MAGISTER TECHNOLOGIAE ENVIRONMENTAL HEALTH: FOOD SAFETY** is my own original and independent research work that is true and authentic. This research work has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for attainment of any degree or qualification.

……..... ……..

KINGSLEY KATLEHO MOKOENA **DATE**

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SUMMARY

Food processing plants and agricultural environments have a long-standing history of being known to provide a conducive environment for the prevalence and distribution of microorganisms which emanate as a consequence of activities undertaken in such premises. Microorganisms in the aforementioned environments may be found in the atmosphere (airborne), and/or on food contact surfaces. Airborne microorganisms from food handlers and in food products and raw materials (as part of bioaerosols) have in the past been implicated as having a potential to cause adverse health effects (especially in indoor environments) and therefore also to have economic implications. Recently their effect on food safety has received increased interest. The recent international interest in bioaerosols in the food industry has played a role in rapidly providing increased understanding of bioaerosols and their effects in different food processing environments. However, there is still a lack of research on the actual impact of bioaerosols over time in most of the food premises especially in Southern Africa and other developing countries.

The overall purpose of this dissertation was to assess possible microbial contaminants and the role of selected environmental parameters on these microbes at a dairy farm plant in central South Africa. In relation to the purpose of the study, the objectives of this dissertation were to investigate and establish the food handler's food safety knowledge, attitude, behaviour and practices. The sub-objective was to investigate the prevalence and distribution of microbial contaminants (both airborne and food contact surface populations), and concomitant environmental parameters. The microbe isolates from both investigations (i.e. air samples and food contact surfaces) were identified to strain level using matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS). The findings of this study in

relation to food handlers' food safety knowledge, attitude, behaviour and practices indicated a dire need for training of employees as well as improved health and hygiene measures as emphasised by some of the identified strains. The environmental parameters (both indoor and outdoor) were similar, with no relationship established between airborne microbes' prevalence and environmental parameters. The samples of the airborne microbial populations in both indoor and outdoor environments were similar. Airborne microbial counts at the dairy farm plant over the entire duration of the study ranged between 1.50×10^{1}cfu.m^{-3}and 1.62×10^{2}cfu.m^{-3}. Microbial counts on food contact surfaces ranged between 2.50×10^{2} cfu.cm^{-2} and 1.10×10^{5} cfu.cm^{-2} over the entire duration of the study. A wide variety of microorganisms (from air and food contact surfaces) such as the Gram-positive bacteria, Gram-negative bacteria, as well as fungi were present at the dairy farm plant. A number of the isolated genera have previously been associated with agricultural environments whilst others are associated with hospital environments. The positively identified strains were from genera such as *Aeromonas, Arthrobacter, Candida, Pseudomonas, Pantoea, Citrobacter, Staphylococcus, Bacillus, Escherichia, Rhodococcus* and *Rhodotorula,* amongst others.

The isolation of microorganisms associated with food spoilage and foodborne disease outbreaks, which are known as indicator organisms such as *Escherichia coli, Staphylococcus* and *Bacillus* from both air and surface samples, signified possible faecal contamination and could be attributed to poor health and hygiene practices at the dairy farm plant. Despite the isolation of microorganisms associated with food spoilage and foodborne disease outbreaks, the isolation of microorganisms not usually associated with the food processing industry (usually associated with hospital environments) was an enormous and serious concern which suggested a need for further investigations at dairy farm plants as the implications of these pathogenic microorganisms in food is not known. The isolation of similar microorganisms from both the air

samples and surface swabs suggests that airborne microbes have a potential of settling on food contact surfaces, therefore having a potential to contaminate dairy products which are known to be more prone to contamination and which, because of their nutritional status, serve as a good substrate for the growth of microorganisms.

CHAPTER 1

General Introduction

AIRBORNE MICROBIOTA AND RELATED ENVIRONMENTAL PARAMETERS ASSOCIATED WITH A TYPICAL DAIRY FARM PLANT: GENERAL INTRODUCTION

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1. 1 GENERAL INTRODUCTION

Food products differ in their biochemical composition; they are also susceptible to contamination and/or spoilage by different microorganisms including airborne microbes. Some of these microbes can play a role in causing foodborne illnesses and foodborne outbreaks. The latter have increased notably over the past two decades in both developed and third-world countries (Rocourt *et al.,* 2003). In recent years, numerous incidents of foodborne diseases have been reported in South Africa (Republic of South Africa: Department of Health, 2007). It therefore becomes important to identify the causes of foodborne illnesses and to recognise contributing practices in food processing establishments (Strohbehn *et al*., 2008).

Food processing is an ancient practice that is still used today to preserve food and to make it safe for human consumption (Macrae *et al.,* 1993; Bernardeau *et al.,* 2006). Food processing is done by making conditions extreme/harsh through denaturation of proteins or by reduction of water content in the food products in order to inhibit microbial growth. In the dairy industry, the shelf-life of milk and milk products is prolonged by the processing and maintenance of cold storage conditions (cold chain). The milk processing industry is one of the leading food industries processing various dairy products and beverages such as milk, yoghurt, cheese and dairy juice products (Belova *et al.,* 1999). In addition, Britz and Robinson (2008) describe the dairy industry as the largest sector in the food-supply chain which also provides ingredients (such as cream, butter, cheese, yoghurt and milk, amongst others) to a number of other food processing sectors. Gerrit (2003) states that the demands of dairy product consumers have led to

the development and revolutionisation of the dairy processing industry. Due to its nutritional quality, milk is prone to microbial contamination and some of the contaminants might be airborne (Salustiano *et al.,* 2003; Nádia *et al.,* 2012). The normal skin flora of a bovine contains opportunistic microorganisms from the environment (soil, water and bedding) and contagious skin sources (mastitis-infected animals) that can infect the teat canal and mammary glands of animals (Oliver *et al.,* 2004). The microbiological infection of mammary glands may result in the inflammation of the udder (mastitis) accompanied by the production of a large number of somatic cells which may contaminate the milk and possibly affect the quality of milk (Gillespie *et al.,* 2009). In addition, this and other available ingredients present a favourable environment for the multiplication of microorganisms in milk (Gilmour and Rowe, 1981; Lues *et al*., 2003).

The presence of airborne microorganisms in food processing plants represents a challenge due to the economic and health problems they may cause, as research has shown that processing plants are prone to indoor air contamination. Shale and Lues (2007) demonstrate that the presence of airborne contaminants can influence the quality of the food products such as red meat, amongst others. Moreover, Jullien and co-workers (2002) report on pathogenic microorganisms' ability to contaminate surfaces as a serious concern in the food industry. Microorganisms are known to settle on and contaminate working surfaces, equipment and the hands of workers, which could lead to contamination of milk and other dairy products (May, 1962; Georgnaras *et al*., 1996; Whyte, 2002; Schlegelová *et al.,* 2010).

Microorganisms can be kept at the lowest possible levels by establishing cleaning programmes in order to keep the factory in a hygienic condition (Gerrit, 2003). However, during cleaning, cleaning agents such as chemicals are used together with water under immense pressure (spraying) and these chemicals may in turn release harmful pollutants which could possibly contaminate the food/beverage products that are produced, adversely affecting the health of employees particularly when personal protective equipment is not used properly. Workers in occupational environments may be exposed to a range of bioaerosols which are associated with a wide variety of health effects (Crook & Sherwood-Higham, 1998; Douwes *et al.*, 2003; Rocourt *et al.,* 2003). To assess hazards and risks, workplace exposure of airborne biological agents in dairy processing must be measured and controlled so that products of highest quality can be produced (Marth and Steele, 1998).

The quality of the air in food processing plants remains a great concern, even though most plants strive to control it. Studies have indicated that air is one of the probable sources of contamination in various food processing environments, including those that process dairy products (Kang and Frank, 1990; Ellerbroek, 1997; Whyte *et al*., 2001; Sutton, 2004; Shale *et al*., 2006). Air is known to contain dust which can comprise of microorganisms and other airborne contaminants which may possibly contaminate food and beverages during processing and packaging (Byrne *et al*., 2008). There is a wide range of airborne contaminants found in food processing environments, but microbial particles are considered more important because of their ability to cause infections,

toxic illnesses and a wide range of allergic responses (Rylander, 1999; Wirtanen *et al*., 2002; Kolk, 2003; Yao and Mainelis, 2006).

Evancho *et al.* (2001) report that the survival and growth of microorganisms in food processing plants can lead to spoilage of finished products. Legislation and consumer pressures mandate that further improvements be made to reduce the pollution potential that may impact on the quality of dairy products. A lack of documented literature on the distribution of bioaerosols has led to the underestimation of their impact on the quality of food products and the health and well-being of humans (Kang and Frank, 1989; Shale and Lues, 2007). Although there are devices that have been developed for the monitoring and analysis of bioaerosols, there is still a lack of data when it comes to the effect of bioaerosols in the food sector. This could be attributed to the lack of agreed standards worldwide.

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CHAPTER 2

LITERATURE REVIEW

AIRBORNE MICROBIOTA AND RELATED ENVIRONMENTAL PARAMETERS ASSOCIATED WITH A TYPICAL DAIRY FARM PLANT: LITERATURE REVIEW

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2.1 FACTORS INFLUENCING MILK QUALITY AND HYGIENE

2.1.1 Definition of milk

Milk is a white, opaque liquid, which can be slightly yellowish in colour (Figure 2.1) and it is excreted by the mammary glands of all female mammals. In the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972, the term "milk" is defined as: "*...the mammary secretion obtained from the mammary glands of healthy cows of the bovine species during the usual lactation period by means of complete and regular milking".* Milk and its products are, and have always been, an integral part of the human diet. Milk is one of the most precious natural materials, serving as a basic food component for humans and most importantly as food for the newborns of both humans and other mammals. Milk is a sweet, highly nutritious food containing a wide range of positive nutritional benefits, which are also generally required by pathogenic and/or spoilage organisms for their own growth, making milk ideal for the survival and proliferation of such organisms (Cawe, 2006; Dairy Standard Agency, 2011). It is because of this that the quality control of milk is regarded as important: the quality of the milk affects the health and well-being of consumers (Cawe, 2006).

Milk contains a variety of nutrients including proteins which are the building blocks of the body, vitamins, fat, carbohydrates and other minerals such as calcium (Harding, 1995). Due to its characteristics and nutritional quality, milk is prone to microbial contamination. From the udder of a healthy cow, milk contains a low microbial load that gets contaminated at various stages of handling and processing (Lues *et al*., 2003).When

Figure 2.1: Sample picture of milk storage in the farm (Adapted from Files world press, 2012)

milk is stored at room temperature its microbial load increases rapidly (Richter *et al.,* 1992). However, the growth or proliferation of microorganisms can be controlled by storing the milk at low refrigeration temperatures, keeping it covered immediately after milking and handling it hygienically (Bonfoh *et al.*, 2003). The hygiene and handling of milk after milking and through all the processing stages is critical in ensuring that milk products of good and acceptable quality are produced. The maintenance of the cold chain is highly significant in preventing an increase of the microbial load and ensuring that milk is processed still in a good and wholesome condition.

2.1.2 Production of milk

Milk is one of the most important beverages that is produced locally, used to feed multitudes of South Africans and in some cases exported. It is the most common source of food in the human diet that is directly available for consumption (Grimaud *et al*., 2009). This has resulted in the dairy industry being described as one of the largest sectors in the food-supply chain which also provides ingredients to a number of other food processing sectors (Britz and Robinson, 2008).

Historically, raw milk in South Africa is, and has always been, produced in the rural areas (farms) and later transported in thermo-regulated tankers to the urban areas (processing plants) where it is processed. A survey done by Banga (2001) indicates a growth in number of smallholding dairy farmers. Technological developments and improvements to milking machines have resulted in the transformation of the dairy

sector (Jansen, 2003), which has resulted in an increase of dairy farmers who process milk at their farms instead of transporting it to dairy plants (Jansen, 2003). On farms, hand milking is the most common method of milking, but this method has shortcomings in that it does not produce enough milk and can have an increased possibility of cross-contamination. Table 2.1 shows the South African National Standards that are applied in the dairy industry in order to ensure the safety of milk and other milk products, and also to ensure longevity of the processed milk products (Republic of South Africa: Department of Health, 1972).

Table 2.1: National Standards applicable to milk in South Africa

Analysis	**Raw milk before further processing**	**Raw milk directly to consumers (public) without processing**	**Pasteurised milk**
Total count	$< 2x10^5$ cfu.ml^{-1}	$< 5x10^4$ cfu.ml^{-1}	$< 5x10^4$ cfu.ml^{-1}
Coliforms	20 cfu.ml^{-1}	< 20 cfu.ml^{-1}	< 10 cfu.ml^{-1}
E. coli	0	0	0
Pathogens	0	0	0

Adapted from: Foodstuffs, Cosmetics and Disinfectant Act (54), 1972 (Republic of South Africa, National Department of Health, 1972)

2.1.3 Microorganisms of concern in dairy processing

The dairy industry is facing escalating environmental challenges and efforts to improve management of dairy farms have reduced the environmental impact on milk production (Powers, 2009). Regulatory and social pressures mandate that further improvements be made to reduce possible pollution that may impact on the quality of dairy products. Lack of documented literature on the distribution of bioaerosols has led to the underestimation of their impact on the quality of food products and the health and well-being of humans in food processing areas (Kang and Frank, 1989; Shale and Lues, 2007). Information in recent studies in South Africa by Pohl *et al*. (2007) on culturable fungi in South African gold mines, Shale and Lues (2007) on an overview of bioaerosols in the food sector and Nkhebenyane (2010) on the distribution of airborne contaminants in hospices, make it clear that the presence of bioaerosols can lead to food deterioration. Kang and Frank (1989) report that it is very important to understand the dynamics of bioaerosols in order to monitor and control their occurrence. With the current challenges of climate change and issues of global warming it also becomes imperative to assess the distribution of bioaerosols in food and beverage industries (Morey, 2010).

Jayarao *et al. (*2006) and Shale and Lues (2007), amongst others, have shown that Gram-positives (*Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes),* Gram-negatives (*Salmonella* spp. *Campylobacter jejuni*, *Shigella* spp., *Escherichia coli* 0157:H7, *Yersinia enterocolitica*) and Fungi (yeast and moulds) amongst others, have been isolated in various food processing sectors. In the

dairy industry, numerous outbreaks of milk-borne diseases have been thought to have been caused by pathogens such as *Salmonella* spp, *Staphylococcus aureus, Escherichia coli, Campylobacter* spp, *Listeria* spp. and *Yersinia* spp. (Bryan, 1983; Vasavada, 1988). Most of these outbreaks occurred from raw milk that was either not pasteurised sufficiently or from post-pasteurisation contamination (Fahey *et al.,* 1995; Jansen, 2003). Airborne microorganisms in the processing environments may occur from activities taking place, people working, the ventilation systems not operating well and many other possible sources. Table 2.2 illustrates common milk-borne microbes and the diseases they cause.

Food handlers are considered the largest contamination source in the food industry as they may directly or indirectly contribute towards the contamination and possible spoilage of the products that are produced and processed. With dairy products being more susceptible to contamination, the health status and personal hygiene level of food handlers is critical to the safety and quality of dairy products. Microorganisms play an important role in the food industry where they could cause disease and subsequent economic losses and illnesses (Rocourt *et al.,* 2003). A number of microorganisms such as *Staphylococcus, Escherichia coli* and *Bacillus* are known commensals of the human skin, hair, intestinal and respiratory tract of humans may be transferred to dairy products during processing and packaging, thus potentially contaminating them.

Table 2.2: Microbial agents causing foodborne disease outbreaks associated with milk products, 1973-2005

Type of milk-borne disease	Causative agent	Disease/disorder
Food infection	*Salmonella typhi* and related species *Shigella dysenteriae* *Streptococcus* sp. (enterococci)	Typhoid, Salmonellosis (food poisoning) Shigellosis (dysentery) Septic sore throat, Scarlet fever, food poisoning
Food intoxication		
Bacterial	*Staphylococcus aureus* *Clostridium botulinum* *Escherichia coli* *Vibrio cholera*	Food poisoning Botulism (food poisoning) Summer diarrhoea Cholera
Fungal	*Aspergillus flavus* Other toxigenic mould sp.	Aflatoxicosis Mycotoxicosis
Toxic-infections	*Bacillus cereus* *Clostridium perfringens*	Food poisoning Gas gangrene
Other milk-borne disorders (uncertain pathogenesis)	*Aeromonas* sp. *Proteus* sp. *Klebsiella* sp. *Pseudomonas* sp. *Citrobacter* sp.	Food poisoning Food poisoning Food poisoning Food poisoning Food poisoning
New emerging pathogens	*Yersinia enterocolitica* *Campylobacter jejuni* *Vibrio parahaemolyticus* *Listeria monocytogenes*	Diarrhoeal diseases Diarrhoeal diseases Diarrhoeal diseases Listeriosis
Other milk-borne diseases		
Bacterial	*Mycobacterium tuberculosis*	Tuberculosis
Milk-borne diseases: Infections, intoxications and toxic-infections		
Bacterial diseases	*Brucella abortus* *Corynebacterium diphtheriae* *Bacillus anthracis*	Brucellosis Diphtheria Anthrax
Rickettsial diseases	*Coxiella burnetti*	Q fever
Viral diseases	Entero viruses Infectious hepatitis virus Tick-borne Encephalitis Virus Foot and Mouth Disease virus (FMD-virus)	Enteric fever Infectious hepatitis Tick-borne Encephalitis Foot and Mouth Disease (FMD)

(Adapted from: Dairy for all, 2011)

The microbiological quality of dairy products is hugely influenced by the initial flora of raw milk, the processing conditions and post-processing contamination, as milk from the udder is believed to have low microbial loads and only becomes contaminated during and after milking as well as during processing (Lues *et al.,* 2003; Islam *et al.,* 2009). In the dairy environment, contamination from the equipment and unclean milk contact surfaces occurs during production (Lehto *et al.,* 2011). Microorganisms may build up on the equipment and milk contact surfaces resulting in the formation of biofilms which may harbour other microorganisms and may be resistant to cleaning and disinfecting agents, potentially resulting in the contamination and cross-contamination of milk and milk products even after pasteurisation (Vlková *et al.,* 2008, Salustiano *et al.,* 2009).

This build-up of microorganisms on equipment and milk contact surfaces is a significant problem in the dairy industry and is the main source of contamination of dairy products that occurs as a result of improper cleaning and disinfection in the processing area (Gibson *et al.,* 1999; Jessen and Lammert, 2003; Simões *et al.,* 2010; Malek *et al.,* 2012). In the dairy industry, biofilms threaten the safety and quality of dairy products, significantly reducing their shelf-life (Chmielewski and Frank, 2003; Salustiano *et al.,* 2009).

2.1.4 Other possible contaminants in the dairy industry

Food production environments are considered critical factors in determining the quality and safety of food products and in recent years, the demand by consumers and retailers for the production of higher quality foods has increased. The dairy industry, which is associated with high-risk foods, is a major food industry that does not only produce dairy beverages but also raw materials for other food industries (Arnold, 2009). In the dairy industry, raw milk is processed through a number of steps such as chilling, pasteurisation and homogenisation, into a variety of milk (both liquid and dried) and milk products such as butter, cheese, ice cream, and yoghurt. Potential sources of contamination include both direct and indirect contact with contaminated water sources, unhygienic processing conditions and environmental surfaces, poor personal hygiene of food handlers, factory design, airborne contaminants, presence of animals and the efficacy of the cleaning procedures (Lehto *et al.,* 2011). It is as a result of the above-mentioned potential contamination sources that the dairy environment is deemed a reservoir for foodborne pathogens (Oliver *et al.*, 2005).

Spore-formers

A spore is a thick-walled reproductive cell that is microscopic and can withstand unfavourable harsh conditions (Setlow, 2007). Spores may be found in premises where extreme moisture is present, such as in dairy plants and any other place that has heat controlling mechanisms such as ventilation systems. Spore-formers are a group of bacteria which form an endospore when they are stressed, sub-lethally injured, or placed in danger in any way. These are particularly important as they have been

proven to survive normal heating processes (Splittstoesser *et al.*, 1998). In the dairy industry, raw milk is known to be the usual source of spore-forming bacteria in processed milk and milk products (Ledenbach and Marshall, 2009). Higher temperatures are therefore recommended for their destruction during food processing. Some spore-forming microorganisms are reported to have aggravated spoilage problems in the beverage industries especially those producing fruit juices (Doyle *et al.,* 1997; Heyndrickx, 2011).

Endotoxins

Endotoxins are potentially toxic substances found inside or on the outer membrane of the cell wall of Gram-negative bacteria and they can be destroyed easily by heat (Rylander, 1999; Todar, 2002; Srikanth *et al.,* 2008). These are lipopolysaccharide or lipo-oligo-saccharide molecules normally present in the water, soil (dust), air and living organisms (Duchaine *et al.,* 2001; Health and Safety Executive, 2003; Bakutis *et al.*, 2004; Yang, 2004; Srikanth *et al.,* 2008). Endotoxins are found in microbes such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria* and *Haemophilus* (Todar, 2002). *Listeria monocytogenes* is the only Gram-positive bacterium that produces endotoxin (Todar, 2002).

Allergens

In the past, a considerable amount of research has focused on allergens (Ren *et al.,* 1999). Allergens include dust from different operations, plants and animals as well as mould spores (Douwes *et al*., 2003; Taylor and Baumert, 2012). Unfortunately, indoor

environments and apartment buildings also harbour their own allergens which can result in allergic reactions if inhaled, ingested, coming into direct contact with sensitive skin, as well as contamination of food and beverages (Sharma *et al.,* 2007). According to Shale and Lues (2007), microorganisms found in indoor environments may cause health effects classified as either infective or allergenic. Certain chemicals and water can also trigger some allergic reactions (Reddy *et al.,* 2012). Air currents can act as a vehicle for movement of these particles and disperse them over great distances depending on their size and other environmental parameters (Douwes *et al*., 2003).

Volatile organic compounds

According to the international performance measurement and verification protocol committee (IPMVP) (2002), volatile organic compounds (VOCs) are a group of gaseous pollutants containing carbon. Volatile organic compounds are considered air contaminants. These VOCs are said to be common emissions from outdoor sources such as motor vehicles, aircrafts, incinerators and food processing operations (US EPA, 2008). Volatile organic compounds can also occur as metabolites that may be produced by microorganisms as well as humans as a by-product of their metabolic reactions. Furthermore, VOCs can result from indoor activities such as cleaning, disinfecting and cooking. The indoor environment has been reported to contain dozens of VOCs at concentrations that can be measureable (IPMVP, 2002). Volatile organic compounds are capable of migrating directly through buildings and as a result they can be found almost everywhere, including in indoor environments. These VOCs are the most prevalent contaminants and as a result of their mobility in the environment, they

are detectable in most media (Hiatt and Pia, 2004). Inside processing plants, vehicles such as fork-lifts that are used to transport processed products from the packaging area to the storage area may also emit VOCs.

In the food industry, volatile organic compounds have been said to be responsible for the off-odours and flavours associated with food spoilage (Zeuthen and Bøgh-Sørensen, 2003). It has been reported that in the past, volatile organic compounds have been detected in cow's milk (Fabrietti *et al*., 2000). Microorganisms in food produce enzymes such as lipases and proteases which are known to be responsible for the breakdown of proteins and fats (Zeuthen and Bøgh-Sørensen, 2003). During this process, organic compounds which may or may not be volatile are released. Volatile organic compounds are usually associated with problems such as production of toxicity, harmful odours and pollution of the air. The interest in VOCs as indoor air pollutants has increased in past years (Hester and Harrison, 1995). Some food manufacturing processes have been said to use products that contain VOCs such as flavourings, dyes, inks, adhesives and other surface coatings (Michigan Department of Environmental Quality (MDEQ), 2009). In beverage processing industries, VOCs can occur as products of combustion during processing and also as a result of further treatment of drinking water before it can be used in the production and processing of beverages (Dauneau and Perez, 1997). Milk from animals is susceptible to potential contamination by organic compounds that are present in the atmosphere, food and water as it cannot be isolated from the environment (Hiatt and Pia, 2004).

2.1.5 Microbiological analysis

A number of sampling and analysis methodologies (biological, physical and chemical) on bioaerosol contamination have been studied and described in a number of scientific papers (Martinez *et al.,* 2004; Cruz and Buttner, 2007; Hameed and Awad, 2007; Wang *et al.,* 2010).

Biological methods based mainly on the microbial particles' biological activity are classical techniques used for the detection and identification of airborne microbes; extensive periods may be required to perform adequate assays for these methods. On the other hand, physical analytical methods used for the detection and identification of microorganisms (including airborne) are relatively rapid and are based on determining the size and shape of microbes. However; they lack specificity (Van Wuijckhuijse *et al.,* 2005). Chemical analytical methods are considered the fastest ways of analysing microorganisms by mass spectrometry. One example of the latter is matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltronics, Germany) (Kim *et al.,* 2005).

MALDI-TOF MS can be used for the analysis and fingerprinting of unknown colonies in order to identify microorganisms (including airborne) such as bacteria and fungi directly, with no need for protein extraction prior to analysis, resulting in real time results and being ideal for the fast-food processing world (Jurinke *et al.,* 2004; Van Wuijckhuijse *et al.,* 2005; Salaun *et al.,* 2010; Wolters *et al.,* 2011).

2.2 BIOAEROSOLS AND ENVIRONMENTAL PARAMETERS

Bioaerosols are airborne microbial contaminants that are ubiquitous in nature and can be detrimental to the health and well-being of humans and animals, as well as the quality and shelf-life of food and beverage products (Kozak, 1988; Robertson, 1998; Wirtanen *et al.,* 2002; Shale and Lues, 2007). Bioaerosols can be introduced into the environment either by people (i.e. activities like coughing, eating, talking, cleaning and sneezing), animals or raw materials used in the production plants (Griffiths and DeCosemo, 1994). On the other hand, environmental (climatic) conditions play a central role in every sphere of human activities and life in general. Any change in the environmental parameters may affect or create an imbalance of the physical environment. Favourable environments for the presence and survival of airborne microorganisms are influenced by meteorological variables such as humidity, temperature and air flow (direction and velocity) which may affect the concentration, dispersion and viability of airborne microbes (Cox and Wathes, 1995; Jones and Harrison, 2004). Geographical location has also been noted as having a great effect on the type of population as well as on the quantity of bioaerosols in the air within indoor environments (Sutton, 2004). Indoor air consists of a variety of bioaerosols both viable and non-viable. Bioaerosols may vary considerably in composition and size, depending on a variety of factors such as the type of microorganism or toxin, and types of particles they are associated with (Maier *et al.*, 2000). Burge (1995) reports that indoor environments have always played a major role in human health as microorganisms survive and multiply within this environment. In addition, Hartung and Schulz (2008) report that air in modern production premises contains a large variety of air pollutants

such as dust, gases, microorganism and endotoxins, all of which may be part of bioaerosols or play a role in their prevalence. Earlier, Kang and Frank (1990) reported that microorganisms use air as their transport medium to contaminate products directly or to contaminate contact surfaces.

The role of bioaerosols in various industrial settings has been well studied in developed countries; however the role of these airborne microorganisms in the South African food industry is poorly understood. Airborne microorganisms in food processing plants are extremely hazardous because of the economic and health problems they may cause, and research has shown that processing plants are prone to indoor air contamination (Ellerbroek, 1997; Whyte *et al*., 2001; Sutton, 2004; Venter *et al.,* 2004; Shale *et al*., 2006, Butler, 2009; Nkhebenyane, 2010; Natasha *et al.,* 2011; Rajasekar and Balasubramanian, 2011). Microorganisms can settle on and contaminate working surfaces, equipment and hands of employees which could possibly lead to cross-contamination of milk and other dairy products. Furthermore, research has shown that air is the probable source of contamination in various food processing environments, including those that process dairy products (Ellerbroek, 1997; Whyte *et al*., 2001; Sutton, 2004; Shale *et al*., 2006). It is important to identify the causes of foodborne illnesses and also to recognise contributing practices in food processing plants. The quality of the air in food processing plants is still a great concern, even though most plants strive to control it through means such as cleaning of ventilation ducts and/or the use of ultra violet light.

Generally, exposure to bioaerosols in an indoor environment could be associated with a range of health effects (Shale and Lues, 2007) as bioaerosols contribute roughly about 5-34% of indoor air pollution (Srikanth *et al*., 2008). In addition, bioaerosols have been reported to lead to both short and long-term adverse health effects such as toxic illnesses, allergies and infections (Burge, 1995; Douwes *et al*., 2003). As a result of their size, bioaerosols can remain airborne for a long time and are capable of migrating through buildings (Cox and Wathes, 1995). Depending on their type and origin, the particle size of bioaerosols may range between 0.01 and 100 microns in aerodynamic diameter (Hirst, 1995). These can be a serious problem in indoor environments, particularly in dairy processing plants where highly perishable products are processed and produced, and they can also affect the health and well-being of occupants in those premises.

2.3 BACKGROUND AND HISTORY OF DAIRY INDUSTRY IN SOUTH AFRICA

South Africa produces a wide variety of beverages which are either used locally or exported. Such beverages include various flavoured soft drinks, fruit juices (both ready to drink and concentrated), soda drinks, mineral water (flavoured and un-flavoured), as well as dairy products such as milk. Milk is one of the most important beverages that is produced locally and used to feed multitudes of South Africans. It is the most common source of food in the human diet that is directly available for consumption (Grimaud *et al*., 2009). Due to its wide use, milk and related dairy products have resulted in the

dairy industry becoming the largest sector in the food-supply chain which also provides nutritional ingredients to a number of other food processing sectors (Britz and Robinson, 2008). Apart from producing dairy products as well as dairy by-products for consumers, retailers and other industries, the dairy industry also markets and transports those products. Bulk tank milk is one of the systems used for the public to access milk at lower cost. Milk is normally produced from a dairy plant, farm, dairy farm or from rural and/or semi-urban areas for consumption and other uses by the public.

2.3.1 Dairy farm

A dairy farm is a place where livestock are kept, raised and maintained for the purpose of milk production. Such agricultural facilities are usually located in the rural areas and in some cases may have crop farming to supply feeds to the livestock. The primary role of dairy farms is to provide raw milk to processors, although currently some dairy farmers process their own milk for selling at local markets and international markets. Traditionally, in South Africa, dairy farms were founded before the 1950s and mainly around the big metropolitan areas such as Cape Peninsula, Durban, Witwatersrand, and other large consumer areas (Terblanche, 2009). Historically, humans have always kept a few animals which they milked to feed their families, either by using the milk as such or by producing cheese, butter, cream and other dairy products. In bygone days dairy farmers used hand-milking techniques to harvest milk from cows and other animals, but modern dairy farmers use sophisticated milking machines to harvest and store milk.

From an environmental point of view, on dairy farms, dust from manure (i.e. organic dust), increased traffic on rural roads, agricultural activities such as livestock and crop farming, feeding and feed handling, barn cleaning and maintenance, milking, and general animal confinement may lead to the presence of microorganisms, allergens and endotoxins which may pose an enormous risk to the safety and quality of dairy products and other food products (Lacey and Lacey, 1964; Donham, 1986; Malmberg, 1990; Arnold, 1999). Unfortunately there is very little data available on the impact of airborne contaminants from dairy farm operations on the safety and quality of dairy products or on human health. For the production of good quality milk and milk products, proper management and good hygiene practices on the farm are highly critical. Milk should be handled in a manner that is hygienically proper to ensure its safety and suitability for its intended use. Recently, a number of dairy farmers have started to understand consumer needs and as a result have started to process and produce milk and milk products onsite, which they then sell to consumers or to established retailers.

2.3.2 Dairy processing plant

A dairy processing plant is a facility that is dedicated to the processing of milk and milk products. Traditionally in South Africa, these processing facilities are usually located in the industrial area of towns or cities, and receive milk from the surrounding dairy farms (producers). In South Africa, the dairy processing industry consists of only a few larger processors who operate nationally and a number of smaller processors operating in specific localised areas (Lacto Data, 2011). A number of processors have laboratories at their processing plants and implement quality improvement procedures such as

Hazard Analysis and Critical Control Points (HACCP) procedures and other food safety systems with the objective of improving the quality and safety of their products (Land O'Lakes International, 2007). However, it has been shown that despite these measures, the final products still become contaminated, posing possible health risks to the consumers (Orefice, 1984; Jouve, 2000; Dioguardi and Franzetti, 2010).

From the dairy farms, raw milk is hygienically handled and transported by means of temperature-regulated tankers to the processing facilities, where it is tested on arrival to check whether it adheres to the requirements before being pumped into bulk tanks through sterile tubes to ensure that no microbial hazards are introduced into the milk. On arrival at the processing plant, the raw milk is stored in bulk tanks, homogenised, pasteurised, packaged and refrigerated before being distributed to retailers where milk and its by-products will be sold to consumers. All these processes are done to make milk and its by-products safe for consumption by consumers. In most cases, the layouts of the dairy processing plant at the rural dairy farm and that of the urban dairy processing facility are similar. However, the difference in the surrounding environment may be significant in determining the airborne contamination potential of the processed dairy products.

2.4 RATIONALE

Food contamination through bioaerosols has long been reported in food processing plants such as pork, poultry (Lutgring, et al., 1997; Venter *et al.*, 2004), beef (Shale,

2004) and dairy (Kang and Frank, 1989; Ren and Frank, 1992). Most processes in different industries generate a wide variety of bioaerosols (Zollinger *et al.*, 2006). These aerosolised particles can contaminate the product through surface contamination or human handling (Heldman, 1974; Salustiano *et al.,* 2003*).* Particle diffusion and air currents distribute the particles throughout the building although their viability and ability to cause negative effects to the product as well as to workers depend on other parameters which include their ability to survive and remain infective in susceptible hosts (Cox and Wathes, 1995).

In South Africa, the research focus has been and still is mainly on air pollution created by various industries generally due to chemicals and as a result, there is lack of research on air contaminants in food environments such as dairy plants. A study done by Shale and Lues (2007) identified a need for further investigations regarding the distribution of bioaerosols in food processing environments especially in developing countries.

The microbial quality of milk is crucial for the production of quality dairy products. Research has shown that bioaerosols may influence the quality of the products (Jullien *et al.,* 2002; Shale and Lues, 2007). Depending on the infrastructure and ventilation system, dairy products can be contaminated by airborne contaminants. Once the milk is contaminated especially after pasteurisation, it could have detrimental effects on

consumers, particularly infants and people with compromised immune systems (Salustiano *et al.,* 2003; Aaku *et al.*, 2004).

2.4.1 Limitations of the study

The initial plan was to conduct this study in all dairy farm plants in the central Free State but due to competition among the companies which produce similar products, this turned out to be unfeasible. As a result, the final decision was made to focus only on one dairy farm plant that was shown beyond reasonable doubt to cover all the dairy farm activities and dairy products produced by their competitors.

2.4.2 Pilot study

A pilot study was conducted in a semi-urban diary plant to test the validity of the questionnaire and sampling methods. The data gathered is attached in the appendix section as this paper will be submitted as a research note due to the data gathered that showed potential for publication (Appendix B).

2.4.3 Study aim

This study focused on the assessment of airborne and surface microbial contaminants and related environmental parameters within a dairy farm plant. For the purpose of this

study, a pilot study was conducted to test the validity of the questionnaire and quantification methods for bioaerosols and environmental parameters.

2.4.4 Objectives of study

The objectives of the study were:

- to quantify and identify airborne microbes outside of and within the dairy farm processing plant;
- to assess the distribution of microorganisms on working surfaces and correlate this with airborne prevalence in the dairy farm plant;
- to evaluate the influence of environmental parameters on bioaerosols within and outside of the dairy farm plant; and
- to collect data on health and hygiene knowledge, as well as production practices during processing in the form of questionnaires and a checklist, in relation to bioaerosols during processing.

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CHAPTER 3

MALDI-TOF MS FINGERPRINTING OF AIRBORNE MICROBIOTA IN A DAIRY FARM PLANT

MALDI-TOF MS FINGERPRINTING OF AIRBORNE MICROBIOTA IN A DAIRY FARM PLANT

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3.1 ABSTRACT

The effect of bioaerosols in the dairy industry is yet to be investigated thoroughly as little is known about the composition of airborne contaminants in the dairy farm plants. This study focused on indoor airborne contaminants as well as the effects of environmental parameters thereof in a central South African dairy farm plant. Simultaneous measurements of bioaerosols, temperature, wind velocity and relative humidity were performed at a dairy farm plant in central South Africa during the dry and wet seasons. Airborne microbes were cultured, quantified and Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF MS) used for fingerprinting of airborne microbes. Average fungal counts in the fresh processing plant were higher (3.06×10^2 cfu.m^{-3}) compared to bacterial counts (1.94×10^2 cfu.m^{-3}). In the ultra-heat treatment (UHT) processing plant, average fungal counts were 6.91×10^2 cfu.m^{-3} while average bacterial counts were 2.57×10^2 cfu.m^{-3}. However, in the outside environment, average bacterial counts were higher (2.67×10^2 cfu.m^{-3}) than fungal counts (5.50×10^1 cfu.m^{-3}). Environmental parameters between indoor and outdoor environments did not vary significantly. Some of the most commonly identified microbiota were *Bacillus* spp, *E. coli*, *Streptococcus* spp, *Candida* spp, *Clostridium* spp, *Acinetobacter* spp., *Staphylococcus* spp, *Arthrobacter* spp, and *Pseudomonas* spp. The identified pathogens raise concern and indicate a dire need for strong hygienic measures.

Keywords**:** dairy farm plant, bioaerosols, environmental parameters, indoor air quality, MALDI-TOF MS, fingerprinting**.**

3.2 INTRODUCTION

The dairy industry is one of the largest leading sectors in the food-supply chain which does not only produce and process milk and milk products, but also provides nutritious ingredients to a number of other food processing sectors (Belova *et al.,* 1999; Britz and Robinson, 2008). Demands for dairy products by consumers have led to the development and revolutionisation of the dairy processing industry (Gerrit, 2003). As a result of its nutritional value, milk and milk products present a good medium for the growth of microorganisms and some may be introduced through air (Salustiano *et al.*, 2003; Frank, 2009).

The quality of air in food processing environments is a great concern as there is a wide range of airborne contaminants found in food processing environments (Kolk, 2003; Yao and Mainelis, 2006). Air has been reported as the probable source of contamination in some food processing environments (Sutton, 2004; Shale and Lues, 2007). Early studies on the enumeration of the microbial populace have been recorded from as early as 1934 (Butler, 2009). Olsen and Hammer (1934) performed a study at dairy plants where they used settling plates to enumerate the numbers of bacteria, yeasts and moulds. In recent years, exposure to bioaerosols in occupational environments has been a subject of concern due to the prevalence of bioaerosols in many of these environments (Jones and Harrison, 2004). However, one challenge has also been the methods used to analyse quantified airborne microbes. The use of methods such as PCR, ELISA and MALDI-TOF MS has been reported in microbial identification and/or fingerprinting but not from air origin.

Gravity, air density and meteorological variables such as humidity, temperature, air flow (direction and speed) amongst other things, play a role in the distribution of airborne microorganisms indoors (Jones and Harrison, 2004; Gilbert and Duchaine, 2009). Both outdoor and indoor air consists of a variety of bioaerosols that are both viable and non-viable. Hartung and Schulz (2008) report that air in modern production premises contains a large variety of air pollutants such as dust, gases, microorganisms and endotoxins, amongst others. The indoor environment has always played a major role resulting in a wide range of health effects and contributing roughly about 5-34% of indoor air pollution (Shale and Lues, 2007; Srikanth *et al*., 2008). In addition, some bioaerosols have been reported to lead to both short and long-term adverse health effects such as toxic illnesses, allergies and infections (Srikanth *et al.,* 2008). These contaminants have been reported also to affect the quality of food products in some cases: this is a field that still requires more research (Lutgring *et al.,* 1997; Venter *et al.,* 2004; Shale and Lues, 2007; Von Tayson, 2009).

Once airborne contaminants are indoors, their dispersal and survival can be influenced by many factors. Climatic parameters such as temperature, relative humidity, wind speed, rainfall, etc., in occupational settings, have been demonstrated to have a seasonal influence on the prevalence and concentration of airborne contaminants (Tiwari, 2006; Shale and Lues, 2007). In food production environments, a strong correlation exists between the efficiency of ventilation systems and the concentration of bioaerosols. This is because ventilation systems can significantly influence the

temperature changes in the indoor environment, impacting on the dispersal, dilution and removal of air pollutants (Venter *et al.,* 2004; Shale and Lues, 2007).

The prevalence of bioaerosols as influenced by environmental factors in food processing plants is extremely hazardous because of the possible economic and health problems they may cause (Ellerbroek, 1997; Whyte *et al*., 2001; Sutton, 2004; Venter *et al.,* 2004; Shale *et al*., 2006, Butler, 2009; Nkhebenyane, 2010; Rajasekar and Balasubramanian, 2011; Natasha *et al.,* 2011). Airborne microorganisms may end up settling on and contaminating working surfaces, equipment and hands of employees which could possibly lead to cross-contamination of milk and other dairy products. Additionally, due to their size, bioaerosols can remain airborne for a long time and are capable of migrating through buildings (Srivastava *et al.,* 2012). It is therefore the aim of this study to quantify and fingerprint bioaerosols using MALDI-TOF MS as well as to assess the role of selected environmental parameters on bioaerosols dispersion within the dairy farm processing sections. This is the first report on the use of MALDI-TOF MS fingerprinting from samples of air origin in the South African food industry. As a result, this study will shed light on the prevalence of known and unknown bioaerosols associated with dairy product processing and also explore the ability of MALDI-TOF MS to rapidly identify airborne microorganisms.

3.3 MATERIALS AND METHODS

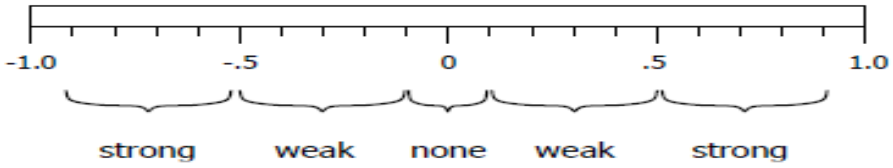
3.3.1 Sampling site

The study was conducted on a 6000 hectare dairy farm that is situated on the northern side of the Free State province in central South Africa. The area is a semi-arid region comprised of general vegetation that is mainly made up of highveld grassland and shrublands, and it is situated at an altitude of approximately 1.395 m above sea level. This dairy farm employs approximately 300 employees in different sections on the farm. Operations on this farm include livestock farming and crop farming (for feed for over 2000 cattles) activities with the processing of dairy products done within the same premises. A floor diagram of the said farm is attached in Appendix A (Figure A1).

Samples were collected throughout production during the dry and wet seasons for possible seasonal variations comparison where necessary (Huang *et al.,* 2002). For every sampling run, at least 6 samples were taken outside i.e. two in the farming area, two outside the UHT plant and the remaining two outside the non-controlled area. Four samples were taken inside the controlled area and six inside the non-controlled area (number of samples is proportional to the size and number of employees). Samples were taken for 10 consecutive sampling cycles with two-week intervals between them to compare both dry and wet seasons for the purpose of the study. The same sampling times and frequency were employed throughout the sampling period for the different environmental parameters concomitant to bioaerosols.

3.3.2 Study design and statistical analysis

For the purpose of this project, descriptive and observational study designs were used, where the prevalence of airborne microbes was determined concomitant to related environmental parameters. All air samples were collected and analysed at least in duplicate and environmental parameters were collected in triplicate. Microsoft Excel 2010 and Sigma Plot 8.1 were used for applicable statistical analysis where necessary. For the correlation coefficient, Taylor (1990) was used for the wording described below (Scheme 1). The correlation *r* value requires both magnitude and direction of either positive or negative. The *r* value ranges between -1 and +1. The *r* values between 0.1 and 0.5 indicate that the relationship is 'weak'. The *r* values between 0.5 and 0.9 indicate that the relationship is 'strong'. The *r* values greater than 0.9 indicate that the relationship is "extremely strong"



Scheme 1: Adapted from Taylor (1990)

3.3.3 Quantification of airborne microbiota

Samples were collected at a height of 1,5m above the floor by means of impaction on soft agar plates. A single stage (SAS Super-90) surface air sampler (PBI International, Milan, Italy) was used for this purpose. The air sampler was calibrated at an airflow rate of 0.03 $m^3.min^{-1}$ and all the detachable parts were pre-autoclaved and disinfected with

70% ethanol between each sample run (Venter *et al.*, 2004; Shale *et al*., 2006; Coccia *et al.,* 2010). Plate Count Agar (PCA) (Merck, South Africa) and Potato Dextrose Agar (PDA) (Merck, South Africa) were used for the quantification of total aerobic count and yeast and moulds respectively. All impacted plates were incubated in an inverted position at standardised, appropriate temperatures and incubation periods (Rajasekar and Balasubramanian, 2011) with all colonies expressed as colony forming units per cubic meter of air sampled.

3.3.4 MALDI-TOF MS fingerprinting

Taxonomic identification and fingerprinting of isolated microorganisms was done by MALDI-TOF MS (Bruker Daltonics, South Africa), which provides protein profiles from each isolate. Briefly, cells (single colonies) from biological material were recovered by scraping the plate and transferring into an Eppendorf tube with 300 *µ*L of Ultrapur water (Merck, SA) and mixed thoroughly. Absolute ethanol (900 *µ*L) was added carefully, mixed thoroughly, and centrifuged at maximum speed (13200 rpm) for 2 minutes at room temperature. The supernatant was decanted and the pellet air-dried at room temperature. The dry pellets were mixed thoroughly by vortexing with 50 *µ*L formic acid (70%) (Merck, SA), followed by the addition of 50 *µ*L pure acetonitrile (Merck, USA) and further mixed thoroughly. The mixture was centrifuged at maximum (13200 rpm) speed for 2 minutes, and approximately 1 *µ*L of the supernatant was placed onto a Micro Scout Plate (MSP) 96 polished steel target plate (Bruker Daltoniks, Germany) and allowed to dry at room temperature. Subsequently, each sample was overlaid with 1 *µ*L of the

HCCA matrix solution (a saturated solution of a-cyano-4-hydroxy-cinnamic acid (Sigma, USA) in 50% acetonitrile-2.5% trifluoroacetic acid) (Bruker Daltronics, Germany) and air dried at room temperature. The analysis of all strains was performed with a Microflex LT mass spectrometer (Bruker Daltonics, Germany) using Flex Control software (Version 3.0, Bruker Daltonics, Germany). The spectra were recorded in the linear positive mode (with the laser frequency of 20 Hz; ion source of 1 voltage, 20kV; ion source of 2 voltage, 18.6 kV; lens voltage, 7.5 kV; mass range, 2000 to 20 000 Da). For each spectrum, 240 shots in 40-shots from different positions of the BTS spot (manual mode) were collected and analysed. The spectra were internally calibrated by using *Escherichia coli* ribosomal proteins as the standard. The raw spectra were imported into the BioTyper software (version 3.0, Bruker Daltonics, Germany), processed by standard pattern matching with standard settings, and the results reported in a ranking table with colour codes. Outcomes of the pattern-matching process were expressed as proposed by MALDI-TOF biotyper (MT) manufacturer with identity (ID) scores ranging from 0 to 3. Scores <1.70 were considered not to have generated a reliable ID; a score of 1.7 <ID <1.9 was considered ID to genus, and a score >1.9 was used for reliable species ID.

3.3.5 Environmental parameters

Temperature, relative humidity and wind velocity were evaluated during dry and wet seasons, and the readings were done in triplicate at a height of 1.5 m above the floor (Venter *et al.,* 2004). The following direct reading instruments were used: 1) Area

tempstress monitor (QUESTemp°32; Quest Technologies Inc., Oconomowac, WI) to measure temperature and relative humidity, and 2) Vane airflow anemometer (Airflow Instrumentation LCA 6000 VT, High Wycombe, Buckinghamshire, UK) (Venter *et al.,* 2004). Pre- and post-calibration of the tempstress monitor was done in order to ensure that the instrument was in a good working state. Positive and negative controls were included and all analysis and assays were repeated at least in triplicate.

3.4 RESULTS AND DISCUSSION

3.4.1 Airborne bacterial counts

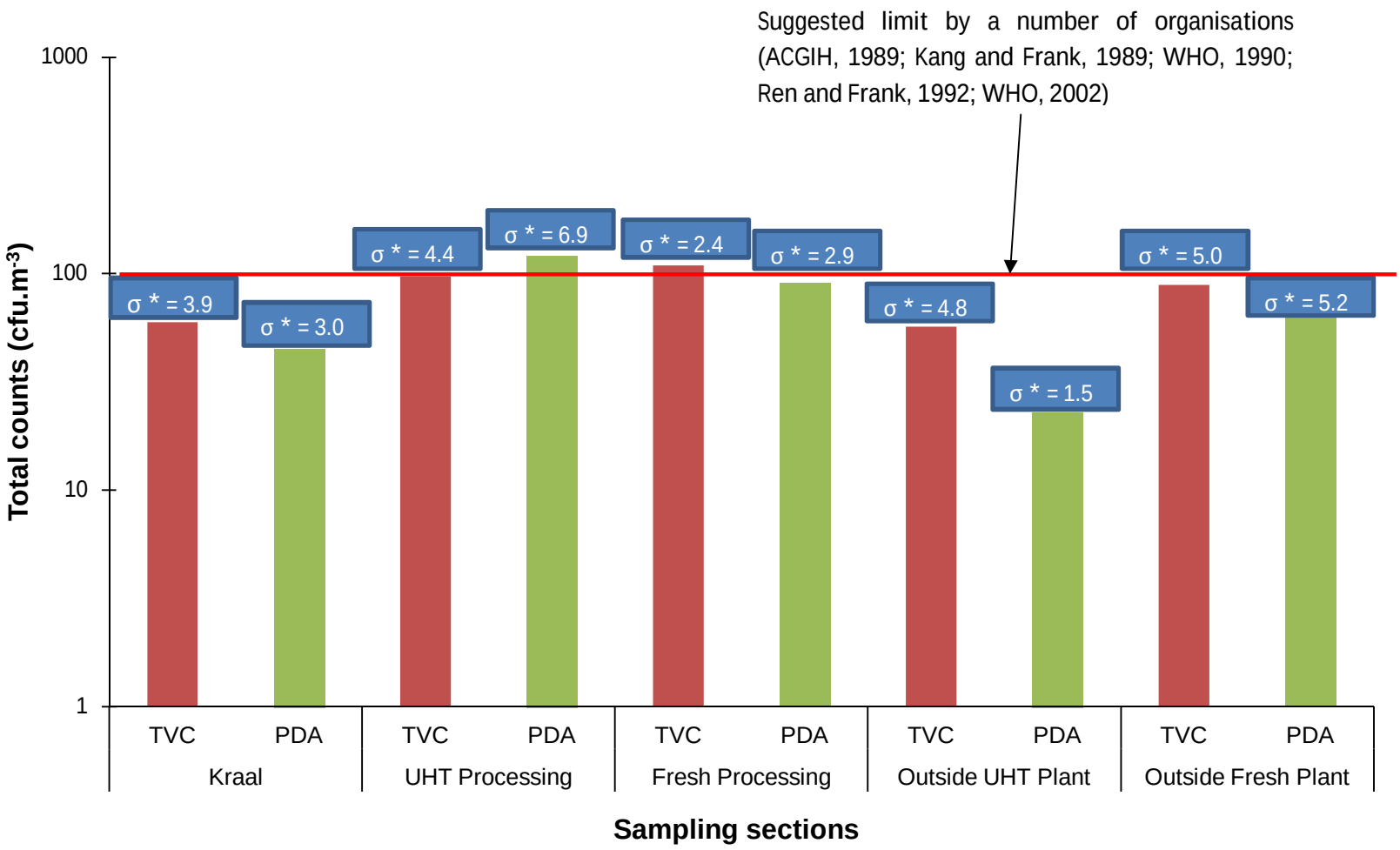
The average concentrations of bacterial counts ranged from 1.50×10^1 to 1.62×10^2 cfu.m^{-3} as depicted in Figure 3.1. In the fresh processing section, the highest counts were 9.1×10^1 cfu.m^{-3} whilst the total counts were 1.091×10^3 cfu.m^{-3} over the duration of the entire sampling period. Outside the fresh processing area, the highest bacterial counts were 1.62×10^2 cfu.m^{-3} with the total counts during the entire study being 8.89×10^2 cfu.m^{-3}. In the UHT processing section, the highest counts were 1.39×10^2 cfu.m^{-3} with the total counts during the entire study being 9.72×10^2 cfu.m^{-3}. The highest counts outside the UHT processing section were 1.52×10^2 cfu.m^{-3} with the total counts during the entire study amounting to 5.69×10^2 cfu.m^{-3} over the duration of the study. All in all, the bioaerosol levels were on aggregate lower than the levels recommended by Kang and Frank (1989) for mesophilic aerobic bacteria of 180-360 cfu.m^{-3}. Bioaerosol levels varied on sampling days, and in some cases levels were lower or higher than the proposed limits by Ren and Frank (1992) in a milk processing plant and lower/higher than a minimum of 100 cfu.m^{-3} as accepted by the American Conference of

Governmental Industrial Hygienists (1989) and the World Health Organisation (1990, 2002). Generally, the results were similar to those found by Salustiano *et al.* (2003) in their study when they reported that microbial counts were between 10 and 1310 cfu.m^{-3} in the air of the dairy processing area. The exposure of immune-compromised people to high levels of airborne bacteria distributed in the breathable air at the dairy farm plant can potentially be associated with respiratory-related diseases, and potential food contamination can result in the spoilage of food (Kim *et al.,* 2010).

3.4.2 Airborne fungal counts

The average concentration of fungal counts ranged from 1.50 x 10^1 to 2.76 x 10^2 cfu.m^{-3} as indicated in Figure 3.1. In the fresh processing section, the highest recorded fungal counts were 1.15 x 10^2 cfu.m^{-3} with the total counts during the entire study being 9.02 x 10^2 cfu.m^{-3}. The highest counts outside the fresh plant were 1.80 x 10^2 cfu.m^{-3} with the total counts during the entire study amounting to 6.93 x 10^2 cfu.m^{-3}. In the UHT processing section, the highest fungal counts were 2.76 x 10^2 cfu.m^{-3} and the total counts during the entire study were 1.21 x 10^3 cfu.m^{-3}. Outside the UHT processing area, the highest counts amounted to 4.5 x 10^1cfu.m^{-3} with the total counts during the entire study being 2.28 x 10^2 cfu.m^{-3}. Human exposure to fungal spores can cause numerous respiratory-related disorders such as asthma, chronic bronchitis and pneumonitis, depending on the susceptibility level and immune system of the exposed individuals (Eduard, 2009; Klarić *et al.,* 2012). In feeds, fungi produce mycotoxins which are considered to be primary agents that cause acute health and/or production

problems in a dairy herd (Magan and Aldred, 2007). Yeasts are used in the daily production of most fermentable foods (such as starter cultures in dairy products); however, their undesired presence in food and feeds is considered to have negative effects as it can result in spoilage (Lind, 2010).



**Standard Deviation (σ)*

Figure 3.1: Average counts of culturable airborne microorganisms isolated within the dairy farm plant

3.4.3 Inter-relationships amongst microbial counts and environmental parameters

In order to determine the exact relationships amongst various microbiota and environmental parameters, Spearman's correlation coefficient and F-Test (two-tailed probability) and Taylor's (1990) defnitions were used to construct a correlation matrix and significant differences. Microbial counts in the fresh plant (area 1 and 2) showed a correlation coefficient between bacteria and fungi to be r= 0.684 and 0.901 respectively. In addition, there was no statistically significant difference between area 1 and area 2 (p= 0.481). On the other hand, there was a negative 'weak' correlation (r= -0.159) between bacteria and fungi in the outside area of the fresh plant. Furthermore, there was a significant difference between area 1 and outside (p= 0.003), as well as a statistically significant difference between area 2 and outside (p= 0.021).

In the UHT plant there was a 'fair' positive correlation (r= 0.523) and a 'strong' positive correlation (r= 0.866) between sampled areas 1 and 2. However, there was a statistically significant difference between area 1 and area 2 of the UHT plant (p= 0.005). Moreover, there was also a 'strong' positive correlation between bacteria and fungi (r=0.632). Furthermore, there was no statistically significant difference between microbial counts of area 1 and the outside (p= 0.945). However, there was a statistically significant difference between area 2 and outside (p= 0.004). There was also a 'strong' positive correlation between bacteria and fungi in the kraal area r= 0.906. Lastly, there was no statistically significant difference between the kraal area and

outside area of the fresh plant (r= 0.089); as well as no statistically significant difference between the kraal area and the outside of the UHT plant (r= 0.699).

With regard to the environmental parameters in the fresh plant processing area, there were 'weak' negative correlations between temperature and relative humidity (r= -0.096), temperature and wind velocity (r= -0.011), and relative humidity and wind velocity (r= -0.476). On the other hand, in the outside area of the fresh plants, there were correlation coefficients between temperature and relative humidity (r= -0.437), temperature and wind velocity (r= 0.137); and between relative humidity and wind velocity (r= -0.409). Interestingly, with regard to the UHT plant processing area, there were 'strong' and 'weak' positive correlations between temperature and relative humidity (r= 0.885), temperature and wind velocity (r= 0.211); and between relative humidity and wind velocity (r= 0.056). Similarly to the former coefficient values of the outside area of the fresh plant, coefficient values of the UHT on the outside were r= -0.043; r=0.151 and r= -0.393 for temperature and relative humidity, temperature and wind velocity, and relative humidity and wind velocity respectively. Finally, there were statistically significant differences between fresh plant processing area and outside the fresh plant (p= 0.005), the fresh processing area and the UHT processing area (p= 0.002) as well as between the fresh plant processing area and the outside UHT plant (p= 0.001). However, there were no statistically significant differences between fresh outside and UHT processing area (p=0.755) as well as between UHT processing area and its outside area (p= 0.498).

3.4.4 Associated environmental (climatic) parameters

This region of central South Africa experiences a semi-arid climate, comprising of hot summer days (average maximum: 32°C, average minimum: 19°C (around January), frequent thunderstorms in the afternoon) and cooler, dry winters (average maximum: 14°C, average minimum: -3°C (around July), often accompanied by frosts). The relative humidity of the region normally ranges between 18% (dry) and 92% (very humid) over the course of the year, and rarely drops below 8% (very dry) and with the possibility of reaching levels as high as 100% (very humid). Wind velocity in the region varies from 0 m/s to 7 m/s over the course of the year.

Historical records indicate that the wind direction trends in the central South African region between 1974 and 2011 over the course of an average year were from the northerly (14%), north-easterly (11%), north-westerly (9%), south-westerly (10%), and westerly (10%) directions (Figure 3.2).

The related climatic parameters for the purpose of this project at the dairy farm plant are presented in Tables 3.2 and 3.3. These climatic parameters data are the average values of 10 sampling periods during which air samples (bioaerosols) were collected. In the fresh processing area, the ambient air temperature ranged from 20.3°C to 25.4°C with an average of 23.7°C (σ = 1.3) during the study. The relative humidity ranged from 39.1 to 82.3% with an average of 62.7% (σ = 12.3) during the study, whilst the wind velocity ranged from 1.3 to 3.2 $m.s^{-1}$ (σ = 0.6). The ambient air temperature outside the

fresh processing plant ranged from 20.3 to 26.5°C with an average of 24.1°C (σ = 1.9). The relative humidity ranged from 11.3 to 60.7% with an average of 29% (σ = 16.7) throughout the study; and the wind velocity ranged from 1.2 to 3.2 $m.s^{-1}$ (σ = 0.7). In the UHT processing plant, the ambient air temperature ranged from 24.4 to 31.1°C with an average of 27.2°C (σ = 2.3) during the study. The relative humidity ranged from 21 to 48.8% with an average of 31.1% (σ = 9.3), whilst the wind velocity ranged from 1.3 to 1.9 $m.s^{-1}$ (σ = 0.2). Outside the UHT processing plant, the ambient air temperature ranged from 22.7 to 25.3°C with an average of 24.3°C (σ = 1.0) throughout the study. The relative humidity ranged from 14.5 to 47% with an average of 27.1% (σ = 10.5), whilst the wind velocity ranged from 1.3 to 3.6 $m.s^{-1}$ (σ = 0.7).

Environmental parameters have been known to have an effect on the prevalence and quantity of airborne microbes. However, this seemed not to be the case during the study. The possible explanation for this could be as a result of environmental variations and different processing activities in both indoor and outdoor environments on the same working day (Salustiano *et al.,* 2003). The prevalence and proliferation of fungi in outdoor and indoor environments depends largely on temperature and the amount of moisture as well as available carbon sources (Malik and Singh, 2004; Mandal and Brandl, 2011). The optimum temperatures for the sporulation growth of fungi is usually around 25-30°C. Temperatures outside the above-mentioned temperature range may have resulted in lower growth and sporulation rates (Sharma and Sharma, 2009; Araujo and Cabral, 2010). Relative humidity (RH) exerts a direct influence on fungal growth and sporulation, and RH levels of between 70% and 100% have previously been

reported to result in high growth and sporulation rates of fungi (Ayyasamy and Baskaran, 2005; Piątkowski and Krzyżewska, 2007). In this study, the low fungal counts could be attributed to the use of air conditioners and mechanised ventilation at the dairy farm plant (Portnoy *et al.,* 2005).

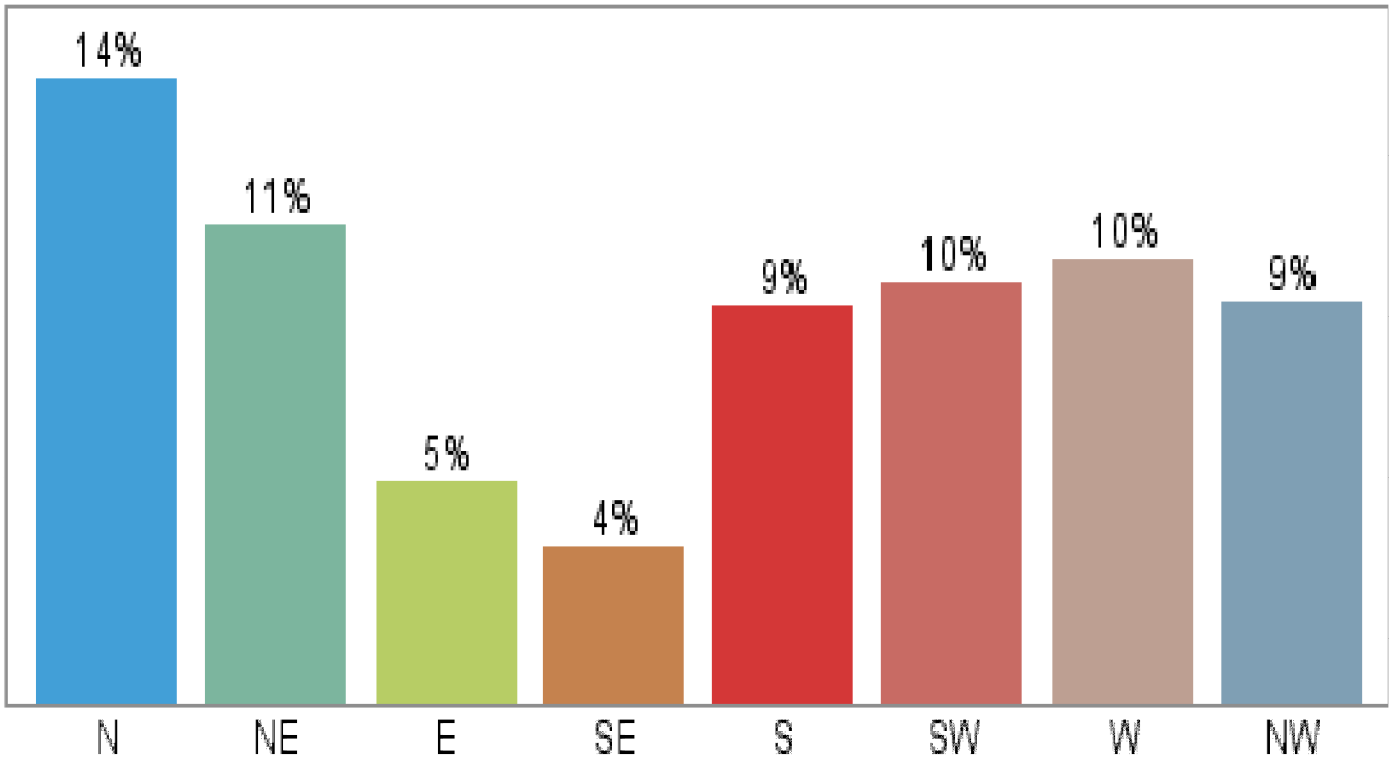


Figure 3.2: Annual wind directions around central South Africa (Adapted from: Weather spark, 2012)

Table 3.1: Detailed environmental parameters expressed as average values for the respective sampling sessions in different sections of the dairy farm plant

SAMPLING SECTIONS						
Fresh plant-processing area				**Outside fresh plant**		
Sample number	**Temperature (°C)**	**Relative humidity (%)**	**Wind velocity ($m.s^{-1}$)**	**Temperature(°C)**	**Relative humidity (%)**	**Wind velocity ($m.s^{-1}$)**
#1	23.1	53.0	2.3	23.8	25.0	2.7
#2	20.3	61.0	3.2	25.5	27.5	2.8
#3	25.1	39.1	2.8	26	11.5	3.1
#4	25.4	60.6	1.3	25.5	25.0	3.1
#5	23.6	49.6	1.6	23.7	11.3	2.1
#6	23.4	65.8	1.6	22	25	3.2
#7	23.6	82.3	1.8	20.3	60.7	1.2
#8	24.0	67.5	2.4	22.3	29	2.7
#9	24.4	77.3	2.4	25.3	59.5	2.0
#10	23.6	70.4	2.8	26.5	15.5	1.2
σ*	**1.3**	**12.3**	**0.6**	**1.9**	**16.7**	**0.7**
UHT plant-processing area				**Outside UHT plant**		
Sample number	**Temperature (°C)**	**Relative humidity (%)**	**Wind velocity ($m.s^{-1}$)**	**Temperature (°C)**	**Relative humidity (%)**	**Wind velocity ($m.s^{-1}$)**
#1	26.0	28.5	1.4	22.9	18.9	2.3
#2	30.6	35.0	1.6	25.0	23.0	3.4
#3	26.3	21.9	1.9	25.1	39.1	2.9
#4	24.4	21.0	1.3	24.4	21.0	3.6
#5	24.5	22.8	1.3	24.0	14.5	3.2
#6	26.5	27.3	1.3	23.0	23.0	3.0
#7	31.1	48.8	1.4	22.7	47.0	1.3
#8	27.9	39.5	1.4	24.8	32.7	2.4
#9	29.0	42.6	1.9	25.3	36.7	2.2
#10	25.6	23.9	1.8	25.3	15.3	1.5
σ*	**2.3**	**9.3**	**0.2**	**1.0**	**10.5**	**0.7**

**Standard Deviation (σ)*

Table 3.2: Average environmental parameters in different sections at the dairy farm plant

	Temperature (°C)				Relative humidity (%)				Wind velocity ($m.s^{-1}$)			
Area	**Min**	**Max**	**Ave**	***σ****	**Min**	**Max**	**Ave**	***σ****	**Min**	**Max**	**Ave**	***σ****
Fresh processing	20.3	25.4	23.7	1.3	39.1	82.3	62.7	12.3	1.3	3.2	2.2	0.6
Outside fresh plant	20.3	26.5	24.1	1.9	11.3	60.7	29.0	16.7	1.2	3.2	2.4	0.7
UHT processing	24.4	31.1	27.2	2.3	21.0	48.8	31.1	9.3	1.3	1.9	1.5	0.2
Outside UHT plant	22.9	25.3	24.3	1.0	14.5	47.0	27.1	10.5	1.3	3.6	2.6	0.7

**Standard Deviation (σ)*

3.4.5 Microbial fingerprinting

Microorganisms play an essential role in the safety and quality of dairy products and dairy farms are believed to be reservoirs for many foodborne pathogens that can cause illnesses through contamination of dairy products and contact surfaces (Salustiano *et al.,* 2003; Oliver *et al.,* 2005). In farm environments, the most important contaminants are bioaerosols (Karwowska, 2005) as microorganisms use air as their transport medium either to contaminate the products directly or to contaminate contact surfaces (Kang and Frank, 1989). The composition of airborne microbiota at the dairy farm plant documented in our study included Gram-negative bacteria, Gram-positive bacteria and fungi, listed respectively in Tables 3.3, 3.4 and 3.5; similar results were also observed elsewhere (Salustiano *et al.,* 2003; Karwowska, 2005; Oliver *et al.,* 2005).

From both outdoor and indoor environments, commonly known food spoilage microorganisms (such as *Acinetobacter* spp, *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus equorum, Listeria ivanovii*) and pathogenic microorganisms (such as *Pantoea* spp, *Aeromonas veronii, Klebsiella pneumonia, Mycobacterium liquefaciens, Acinetobacter* spp, *Enterococcus faecium, Clostridium* spp, *Raoultella ornithinolytica, Streptococcus parauberis, Streptococcus sanguinis, Rhodococcus ruber*) were some of the species isolated from the culturable airborne samples at the dairy farm plant.

Most of the aforementioned species had previously been isolated from a variety of sources including soil, dust, human and animal skin flora, water sources and clinical specimens (Tables 3.3, 3.4 and 3.5); they were however more prevalent at a dairy farm plant. The most typical bacterial strains found in the indoor environments are representatives of the *Acinetobacter, Bacillus, Kocuria, Microbacterium, Pseudomonas* and *Staphylococcus* (Mandal and Brandl, 2011). The fungal isolates comprised of *Aspergillus, Candida and Penicillium* species. The effect of some of the species isolated at the dairy farm environment in terms of health implications has been studied extensively; however the relationship between some of the species isolated which are not usually associated with food and their prevalence at the dairy farm plant is yet to be established and understood.

3.4.5.1 Gram-negative isolates

At the dairy farm plant, pathogenic Gram-negative bacteria from the environment can affect the safety and quality of dairy products through airborne contamination. The results of this study showed that Gram-negative bacteria isolated from the air samples at the dairy farm plant included a high proportion of genus *Acinetobacter, Aeromonas, Citrobacter, Klebsiella, Pantoea, Pseudomonas* and *Raoultella* (Table 3.3). Apart from adversely affecting the quality and safety of food products, the aforementioned genera have a long history of causing infections in both human and animals. In the current study, Gram-negative bacteria that are normally associated with human infections at hospitals were isolated at the dairy farm environment which is a food processing environment.

Aeromonas, *Citrobacter* and *Raoultella* are but a few of the most concerning microbes found in this environment. *Aeromonas* is a genus of Gram-negative rods that are widely distributed in nature from environmental sources such as soil, water sources, sewage, and food samples (Pin *et al.,* 1994). Some *Aeromonas* species can cause human infections in both immune-compromised and immune-competent patients (Janda and Abbott, 1998). In the current study, *Aeromonas veronii* strains (*CECT 4199 DSM*) were isolated from the culturable airborne samples from the dairy farm plant (Table 3.3). These species are commonly found in water sources where there are animals and can be pathogenic in humans, causing diseases such as wound infections, diarrhoea and septicaemia (Hickman-Brenner *et al.,* 1988). Foodstuffs such as organic vegetables and frozen fish have previously been reported to be contaminated with *Aeromonas veronii;* therefore *Aeromonas veronii* has a potential to cause illness in patients who consume contaminated food (McMahon and Wilson, 2001; Castro-Escarpulli *et al.,* 2003). Although *Aeromonas* species have been previously linked with food, there are no existing reports or cases linking them with dairy products.

Secondly, *Citrobacter* species are a group of ubiquitous Gram-negative bacteria that are commonly found in soil, water, sewage, human and animal faecal matter as well as in foods. These species are part of the normal flora of the gastrointestinal tract in both humans and animals. *Citrobacter* species have previously been found in vegetables, fish and dairy products. The genus is commonly used to indicate the general hygiene status in food processing plants. *Citrobacter* species are infrequent opportunistic pathogens in both humans and animals. In humans most infections are nosocomially

acquired and occur mostly in immune-compromised patients, including post-surgery patients. *Citrobacter freundii* strains (*22054_1 CHB; 13158_2 CHB; DSM 15979 DSM; DSM 30039T HAM*) were the only isolated strains of the entire genus (Table 3.3). *Citrobacter freundii* is an opportunistic pathogen that is responsible for infections in immune-compromised people (Puchenkova, 1996).

Raoultella is a genus of oxidase-negative, aerobic, capsulated, non-motile, facultative anaerobic rods from the family of *Enterobacteriaceae*. From the current study, *Raoultella ornithinolytica* (*MB_18887 CHB*) strains were positively isolated (Table 3.3). *R. ornithinolytica* (formerly known as *Klebsiella ornithinolytica*) species are known for the role they play in fish poisoning although they may also cause infrequent and spontaneously occurring bacteraemia as well as enteric fever-like syndromes (Morais *et al.,* 2009). *R. ornithinolytica* has frequently been isolated from estuarine water, fish, termites and ticks (Henriques *et al.,* 2006; Kamanda *et al.,* 2007)

3.4.5.2 Gram-positive isolates

The isolation of Gram-positive bacteria in different food processing environments is not new as they have previously been isolated in bovine, poultry, swine and dairy environments (Matković *et al.,* 2007; Shale and Lues, 2007). The Gram-positive bacteria isolated in this study were predominantly from the genii *Arthrobacter, Agromyces, Bacillus, Clostridium, Enterococcus, Kocuria, Listeria, Staphylococcus, Streptococcus, Rhodococcus, Microbacterium* and *Solibacillus* (Table 3.4).

Table 3.3: MALDI-TOF MS fingerprinted airborne culturable Gram-negative strains at the dairy farm plant

ISOLATED SPECIES	COMMON SOURCE	IMPLICATIONS	REFERENCE
Acinetobacter baumannii ATCC 19606	Soil, foods (vegetables, meat and fish), Hospital environments and water sources	Nosocomial pneumonia infections, Skin colonisation	Dorsey *et al.*, 2004
Acinetobacter bouvetii DSM 14964T DSM	Soil/dust, clinical specimens	Nosocomial infections	Carr *et al.,* 2003
Acinetobacter calcoaceticusB388 UFL	Soil/dust, water sources and faecal matter	Fatal pneumonia	Bouvet and Grimont, 1986
Acinetobacter gerneri DSM 14967T HAM	Activated sludge plants	Not reported	Carr *et al.,* 2003
Acinetobacter johnsonii DSM 6963T HAM	Human skin and mucous membrane, faecal matter, soil (dust) and waste water	Vascular catheter-related bloodstream nosocomial infections	Seifert *et al.,* 1993
Acinetobacter lwoffii 2_Ring 240 MHH *Acinetobacter lwoffii 13 PIM*	Normal flora of the skin, oropharynx and perineum of healthy individuals Stagnant water sources, soil (dust)	Nosocomial pneumonia in immune-compromised people Responsible for community-acquired meningitis and pneumonia via airborne transmission	Bouvet and Grimont, 1986
Acinetobacter parvus DSM 16617T HAM	human and animal non-sterile body sites, and from ear of a dog	Nosocomial infections	Nemec *et al.*, 2003
Acinetobacter sp Genospecies 3 Serovar 3 DSM 9307	Widely distributed in nature, and hospital environments	Food spoilage, nosocomial infections	Skerman *et al.,* 1980
Acinetobacter schindleri DSM 16038T DSM	Human skin, urine, throat	Oil-degrading organisms	Nemec, 2000
Aeromonas veronii CECT 4199 DSM	Soil, animals, water systems	Diarrhoea, wound infections and septicaemia in immune-compromised people	Hickman-Brenner *et al.,* 1988
Arcanobacterium pyogenesDSM 20630T DSM	Normal inhabitant of the mucous membranes of domestic animals Commonly found in bacteria infected wounds Soil	Causes mastitis in cattle Produces suppurative lesions in any organ or tissue in animals	Jurado *et al.,* 2005
Burkholderia tropica DSM 15359 HAM	Crops	Causes diseases in humans, animals and plants	Reis *et al.,* 2004
Citrobacter freundii 22054_1 CHB *Citrobacter freundii 13158_2 CHB* *Citrobacter freundii DSM 15979 DSM* *Citrobacter freundii DSM 30039T HAM*	Widely distributed on plants and in soil, water and the intestines of humans and animals	Increasingly important pathogen in food Potential to colonise humans	Badger *et al.*, 1999
Citrobacter braakii 9314_2 CHB	Widely distributed on plants and in soil, water and the intestines of humans and animals	Increasingly important pathogen in food Potential to colonise humans	Dhouib *et al.*, 2003
Escherichia coli DH5alpha BRL *Escherichia coli RV412_A1_2010_06a LBK* *Escherichia coli ESBL_EA_RSS_1528T CHB* *Escherichia coli MB11464_1 CHB*	Intestines of warm blooded organisms	Food poisoning; food product recalls; foodborne illnesses	Martinez-Murcia *et al.,* 1999
Pantoea sp110 PIM	Soil, water, seeds, animal and human wounds, blood and urine	Opportunistic human pathogen	De Champs *et al.*, 2000
Raoultella ornithinolytica MB_18887 CHB	Infected root canals, gut of fish, ticks, and termites and from estuarine water	Food poisoning, pancreatitis and bacteraemia in humans and mastitis in dairy cows	Morais *et al.,* 2009
Rhizobium rhizogenes B166 UFL	Soil	Plant diseases	Gafni and Levy, 2005
Pseudomonas aeruginosa ATCC	Widely distributed in nature particularly	Food spoilage; causes disease in	Hare *et al.,*

27853 CHB	in moist environments (hospital) and in antiseptic solutions	animals and humans	2012
Pseudomonas koreensis 037_W01 NFI	Agricultural environments (soil (dust))	Not reported	Kwon *et al.,* 2003
Pseudomonas oryzihabitans DSM 6835T	Moist hospital environments, soil (dust)	Opportunistic pathogen of humans and warm-blooded animals	Decker *et al.,* 1991
Pseudomonas taetrolens LMG 2336T HAM	Eggs, milk and various foods	Food spoilage	Spanswick, 1930
Pseudomonas trivialis DSM 14937T HAM	Phyllosphere of grasses	Plant pathogen	Behrendt *et al.*, 2003
Pseudomonas stutzeri 040_W09 NFI *Pseudomonas stutzeri B367 UFL*	Soil (dust), water sources	Opportunistic pathogen	Lalucat *et al.,* 2006
Sphingomonas paucimobilis DSM 1098T HAM	Soil, water, clinical and laboratory equipment in hospitals	Pathogen associated with sporadic or community-acquired infections and sporadic or community-acquired infections	Yabuuchi *et al.*, 1990

The genus *Bacillus* is one of the most ubiquitous bacterial genera of spore-formers. It is immeasurably complex and genetically diverse, comprising of approximately 70 species, some of whose genomes have been thoroughly and completely examined, with new species continuing to be discovered and described (Logan and Turnbull, 2003). However; there is still a lack of data on *Bacillus* species occurring in the environment. In the literature, MALDI-TOF MS has been shown to have the ability to identify, characterise and distinguish different *Bacillus* species and strains (Hathout *et al.,* 1999; Gebhardt *et al.,* 2002; Vater *et al.,* 2002; Pittenauer *et al.,* 2006). From this study, strains from *Bacillus* food pathogens such as *Bacillus subtilis* (*DSM 10T DSM*; *DSM 5660 DSM), B. lichenformis* (*DSM 13T DSM; 992000432 LBK;CS 54_1 BRB*), *B. cereus* (*4080 LBK; 994000168 LBK; DSM 31T DSM*), and *B. sonorensis* (*DSM 13779T DSM*) were positively isolated. These pathogens are naturally present in the soil (dust) and plants, and their presence at the dairy farm plant did not come as a surprise as the environment is conducive to their presence (Labots *et al.,* 1965; Chistiansson *et al.,* 1999).

Streptococcus species on the other hand are Gram-positive bacteria that are commonly commensals of the skin, intestinal tract, mouth and upper respiratory tract of humans. Species from this group are known to cause diseases such as endocarditis, meningitis, bacterial pneumonia and erysipelas in humans, as well mastitis in cattle and streptococcosis in fish (Fernández-No *et al.,* 2012). However, a few *Streptococcus* species which produce lactic acid are deemed beneficial in the dairy industry as they are commonly used in the production of yoghurt, cheese and buttermilk. The lactic acid

produced drops the pH in the dairy products, thereby inhibiting growth of unwanted microorganisms (Garbutt, 1997); it also gives flavour to the products. Strains from *Streptococcus sanguinis* (*DSM 14617T DSM*) and *Streptococcus parauberis* (*DSM 6631T DSM*) were isolated from the culturable airborne samples from the dairy farm plant (Table 3.4).

Staphylococcus is a Gram-positive genus of spherical bacterial species that are non-motile and part of the normal skin flora and upper respiratory tract in both human and animals. Dairy cattle which are affected by mastitis may also be the source of *Staphylococcus.* Staphylococcal species are also widely distributed in most environments and as a result their total eradication is unfeasible. As a consequence of their ubiquitousness, their presence in foods is inevitable and may result in food poisoning as a result of the enterotoxin-producing cocci. Pathogenic *Staphylococcus* species are opportunistic and cause illness in immune-compromised people. Staphylococci species are amongst the most important disease-causing species in both humans and animals. From the current study, a number of Staphylococci strains (*Staphylococcus aureus ssp aureus* (*DSM 20491 DSM*), *Staphylococcus cohnii ssp cohnii* (*DSM 20260T DSM, DSM 20261 DSM*), *Staphylococcus equorum ssp equorum* (*DSM 20674T DSM, DSM 20675 DSM*), *Staphylococcus haemolyticus* (*10024 CHB*), *Staphylococcus hominis ssp novobiosepticus* (*DSM 15614T DSM*), *Staphylococcus hominis ssp hominis* (*DSM 20330 DSM*), *Staphylococcus epidermis* (*6b_S ESL*), *Staphylococcus saprophyticus ssp bovis (DSM 18669T DSM*), and *Staphylococcus succinus ssp succinus* (*DSM 14617T DSM*)) were isolated from the culturable airborne

samples from the dairy farm plant (Table 3.4). The main agent of staphylococcal food poisoning is *Staphylococcus aureus;* however, other *Staphylococcus* species are also involved in causing gastroenteritis amongst other illnesses (Angellilo *et al.,* 2000).

Despite the frequent isolation of the aforementioned strains in different food processing settings, their pathogenic status as bioaerosols has yet to be clearly established (Shale and Lues, 2007). Currently, airborne microbial contaminants may be of more significance than previously recognised, particularly in food-processing environments, mainly because of a lack of information regarding the effect of bioaerosols in food and also because of the ability of air to transport and further disperse airborne microbial contaminants in the food processing area, which may be spoilage and/or pathogenic microbes (Cundith *et al.,* 2002).

3.4.5.3 Fungal isolates

In farm environments, animals and humans are often exposed to high fungal concentrations present in the air (Skaug *et al.,* 2001). The main source of fungi in indoor environments is outdoor air. The prevalence and concentrations of fungi in the indoor environments follow outdoor air seasonal fluctuations (Li and Kendrick, 1995; Lee *et al.,* 2006). Isolated fungal strains of importance in both indoor and outdoor air samples include *Aspergillus, Penicillium* and *Candida* (Gorny *et al.,* 1999; Zorman and Jersek, 2008).

Table 3.4: MALDI-TOF MS fingerprinted airborne culturable Gram-positive bacterial strains at the dairy farm plant

ISOLATED SPECIES	COMMON SOURCE	IMPLICATIONS	REFERENCE
Agromyces neolithicus HKI 321 HKJ	Soil (dust)	Not reported	Jurado *et al.,* 2005
Arthrobacter arilaitensis DSM 16368T DSM	Surfaces of cheese	Not reported	Irlinger *et al.*, 2005
Arthrobacter castelli DSM 16402T DSM	Mural paintings and ceilings	Not reported	Heyrman *et al.,* 2005
Arthrobacter chlorophenolics DSM 12829T DSM	Soil (dust), sewage	Degrade high concentrations of para-substituted phenols	Westerberg *et al.,* 2000
Arthrobacter gandavensis DSM 15046T DSM	Animals, soil (dust), human blood cultures	Mammary and uterine infections	Storms *et al.,* 2003
Arthrobacter oxydans DSM 20119T DSM *Arthrobacter oxydans IMET 10684T HKJ*	Soil (dust), air	Opportunist pathogen in immune-compromised patients	Wauters *et al.,* 2000
Arthrobacter polychromogenes DSM 20136T DSM	Soil (dust), air	Not reported	Huang *et al.,* 2005
Arthrobacter sp B514 DSM 20389 UFL *Arthrobacter sp DSM 20125_DSM* *Arthrobacter sp DSM 20144_DSM*	Soil (dust), air	Microbial degradation of the sodium acrylate oligomer; rarely cause disease in humans	Hayashi *et al.,* 1993; Funke *et al.,* 1996
Bacillus cereus 4080 LBK *Bacillus cereus 994000168 LBK* *Bacillus cereus DSM 31T DSM*	Soil, plants, grains, fruits, vegetables, human nasal tract	Food spoilage and short shelf-life	Kramer and Gilbert, 1989; Todar, 2000
Bacillus drentensis DSM 15600T DSM	Grassland soil	Not reported	Heyrman *et al.,* 2004
Bacillus licheniformis DSM 13T DSM *Bacillus licheniformis 992000432 LBK* *Bacillus licheniformis CS 54_1 BRB*	Soil (dust), raw milk, plant materials and also from almost everywhere in nature due to its highly resistant endospores	Food poisoning and food spoilage (known for contaminating dairy products). Septicaemia in human from consumption of contaminated food	Daffonchio *et al.* 1998
Bacillus megaterium DSM 32T DSM	Soil (dust), plant, water	Opportunist pathogen in immune-compromised patients Produces the penicillin amidase that is used to making penicillin	Eppinger *et al.,* 2011
Bacillus safensis CIP 109412 CIP	Spacecraft and assembly facility surfaces	Not reported	Satomi *et al.,* 2006
Bacillus simplex CS 206_1aI BRB	Soil (dust), air, mural paintings	Pathogenic to insects	Priest *et al.*, 1988
Bacillus sonorensis DSM 13779T DSM	Soil (dust), bread, gelatine extracts and traditionally fermented soya bean paste sauce	Food contamination	Palmisano *et al.* 2001
Bacillus subtilis ssp subtilis DSM 10T DSM *Bacillus subtilis ssp subtilis DSM 5660 DSM*	Soil (dust), air, plant, water, temporary inhabitant of human skin and gastro-intestinal tract, faecal matter, fermented food products	Supports plant growth, restores healthy bacterial communities in the body enhancing one's immune system Food pathogens	Nakamura *et al.*, 1999
Bacillus megaterium DSM 32T DSM	Soil (dust), air, decaying material,	Considered agents of unwanted decay and decomposition in	Skerman *et al*., 1980

		whatever they contaminate. Pathogenic in animals and occasionally isolated in human infections. However; considered not to be pathogenic in humans	
Dermacoccus nishinomiyaensis DSM 20448T DSM	Mouth and skin of mammals and water	Not reported	Stackebrandt *et al*., 1995
Enterococcus faecium 11037 CHB	Human skin	Wounds	Trofa, 2008
Clostridium chauvoei 1024_NCTC 8596 BOG	Soil (dust), manure, water, and the intestinal tracts of humans and animals	Causes severe inflammation of skeletal and cardiac muscle, severe systemic toxicity and high mortality in cattle and sheep (blackleg).	Bagge *et al*., 2009
Clostridium bifermentans 2273_CCUG 35297 BOG	Soil (dust), faecal matter, and sewage	Gas gangrene; humans suffer metastatic osteomyelitis involving the sacrum, spine, and ribs	Scanlan *et al.,* 1994
Corynebacterium xerosis DSM 20743T DSM	Widely distributed in nature. Found in soil, water, plants, food products as well as in the mucosa and normal skin flora of humans and animals	Causes bacteraemia, skin infections, pharyngitis and pneumonia in immune-compromised hosts	Skerman *et al*., 1980
Curtobacterium flaccumfaciens pvar poinsettiae DSM 20149 DSM Curtobacterium albidum HKI 11500 HKJ	Soil, plants	Causes plant diseases and septic arthritis in human	Camara, 2009 Skerman *et al*., 1980
Klebsiella pneumoniae ssp pneumoniae 9295_1 CHB	Normal flora of the mouth, skin and intestines	Opportunistic pathogens in nosocomial infections	Sabota *et al.,* 1998
Kocuria rhizophila DSM 11926T DSM	Soil (dust), mammalian skin, fermented foods, clinical specimens, fresh water source and marine sediments	Opportunistic pathogen in immune-compromised patients causing meningitis, pneumonia and septic arthritis	Takarada *et al*., 2008
Kocuria carniphila DSM 16004T DSM	Meat	Not reported	Tvrzova *et al*., 2005
Kocuria rosea IMET 11363T HKJ	Wide-spread in nature and commonly found in soil (dust), air and water, as well as a normal flora of skin, mucosa and nasopharynx of human and mammals	Causes opportunistic infections in immune-compromised patients such as meningitis, pneumonia and catheter-related bacteraemia	Stackebrandt *et al.,* 1995
Listeria ivanovii ssp *ivanovii DSM 20750T DSM*	Soil (dust), water source, effluents, foods, faecal matter	Food spoilage, potential pathogen	Domínguez-Bernal *et al*., 2006
Macrococcus caseolyticus DSM 20597T DSM	Animal skin and food products (milk and meat)	Human infections	Kloos *et al*., 1998
Staphylococcus aureus ssp aureus DSM 20491 DSM	Faecal matter, foods, soil, normal flora of human intestines	Food poisoning and variety of diseases	Ramesh *et al*., 2012
Staphylococcus cohnii ssp cohnii DSM 20260T DSM *Staphylococcus cohnii ssp cohnii DSM 20261 DSM*	Human skin	Opportunistic pathogen for humans causing different diseases	Kloos and Wolfshohl, 1991
Staphylococcus chromogenes DSM 20454T DSM	Frequently isolated from the skin of pigs and cows and can be recovered from the milk of cows with mastitis	Causes mastitis in dairy animals	Hajek *et al.,* 1986
Staphylococcus epidermis 6b_S ESL	Human skin	Endocarditis in immune-compromised patients	Flannigan, 1992

Staphylococcus equorum ssp equorum DSM 20674T DSM *Staphylococcus equorum ssp equorum DSM 20675 DSM*	Human and animal skin, fermented foods	Food spoilage	Schleifer *et al.*, 1985
Staphylococcus haemolyticus 10024 CHB	Human skin	Septicaemia, peritonitis, urinary tract infections	Gunn and Davis, 1988
Staphylococcus hominis ssp novobiosepticus DSM 15614T DSM *Staphylococcus hominis ssp hominis DSM 20330 DSM*	Human and animal skin	Sepsis, bacteraemia in immune-compromised	Kloos *et al.*, 1998
Staphylococcus saprophyticus ssp bovis DSM 18669T DSM	Associated with domestic animals; carcasses of dead animals	Urinary tract infections	Hajek, 1986
Staphylococcus succinus ssp succinus DSM 14617T DSM	Foods such as cheese and sausages. The skin of healthy wild animals	Not reported	Lambert *et al.,* 1998
Streptococcus parauberis DSM 6631T DSM	Animals, milk, olives	Causes mastitis in cattle and streptococcosis in fish	Fernández-No, 2011
Streptococcus sanguinis	Healthy human mouths and blood stream	Damages heart valves, bacterial endorcatis	Yamaguchi *et al.*, 2006
Rhodococcus ruber DSM 43560 DSM	Soil (dust), water	Opportunistic human pathogen	Gibson *et al*., 2003
Microbacterium sp DSM 15461 DSM	Milk	Not reported	Collins *et al*., 1983
Microbacterium liquefaciens HKI 11374 HKJ	Milk, cheese	Not reported	Collins *et al*., 1983
Microbacterium oxydans DSM 20578T DSM	Air	Not reported	Schumann *et al.,* 1999
Solibacillus silvestris DSM 12223T DSM	Plants	Not reported	Krishnamurthi *et al.,* 2009

In the food industry, yeasts and moulds can play both a beneficial role and also have a negative effect on the food, particularly in fermented products (Ikalafeng, 2008). Yeasts are used in the fermentation of alcoholic beverages, bread and other food products. However, on the negative side, yeasts may result in the spoilage of food products. The most important genus of yeast which is commonly implicated as the major cause of human infections is *Candida* (Moretti, 2007). *Candida* spp. are present in plant debris and soils, and their presence is often associated with the spoilage of foodstuffs including dairy products (Casey and Dobson, 2003; Fitzgerald *et al.,* 2004). At the dairy farm plant, strains from *Candida parapsilosis* (*ATCC 22019 THL), Candida_krusei[ana] (Issatchenkia_orientalis[teleo]) (ATCC 14243 THL), Candida lamblica[ana] (Pichia_fermentans_ssp_fermentans[teleo]) (CBS 603 CBS),* and *Candida_lambica[ana] (Pichia_fermentans[teleo]) (DSM 70090 DSM)* were positively identified (Table 3.5).

Spores of *Aspergillus* and *Penicillium* are responsible for a great deal of food spoilage (Adams and Moss, 2008). *Penicillium* spp. can be found in soil and plant debris, and the farm environment is an ideal place for their presence. *Penicillium* spp. are valuable to humans due to their usefulness in the production of antibiotics and blue cheese. However, a number of species are considered important spoilage organisms of which some can also result in the production of potent mycotoxins (Doyle, 2007). Mycotoxins are secondary toxic metabolites that are produced by many filamentous fungi and are undesirable in food products due to their ability to cause illnesses in consumers (Westby *et al.,* 1997; Kumar *et al.,* 2008; Pietri *et al.,* 2009). In both humans and

animals, mycotoxins may cause damage in a variety of ways including: cytotoxic, estrogenic or teratogenic, immunosuppressive, neurotoxic, mutagenic as well as carcinogenic effects (Bennet and Klich, 2003). Some *Penicillium* species have a potential of spoiling crops and attacking processed as well as refrigerated foods, resulting in enormous financial losses in the food industry (Doyle, 2007). From the current study, strains of *Penicillium chrysogenum (DSM 895 HED)* were positively identified (Table 3.5). *Aspergillus* is a mould that grows fast and the spores are resistant to high temperature which can be a serious concern in the dairy industry. They can spoil a great variety food and non-food items such as paper and grains which should be a concern for dairy farmers who store grains as part of their animal feeds (Doyle, 2007).

Table 3.5: Identified airborne culturable fungal species in the dairy farm plant

ISOLATED SPECIES	COMMON SOURCE	IMPLICATIONS	REFERENCE
Aspergillus fumigatus wild VML	Soil (dust) and decaying matter	Various diseases in immune compromised individuals	Arruda *et al.,* 1990
Candida parapsilosisATCC 22019 THL	Domestic animals, insect, soil (dust)	Septicaemia in immune-compromised patients, nosocomial infections	Trofa, 2008
Candida_krusei[ana]# (Issatchenkia_orientalis[teleo]) ATCC 14243 THL	Seeds of cacao plant	Emerging fungal nosocomial pathogen	Abbas, 2000
Candida lamblica[ana] (Pichia_fermentans_ssp_fermentans[teleo]#) CBS 603 CBS Candida_lambica[ana] (Pichia_fermentans[teleo]#) DSM 70090 DSM	Soil (dust), dairy products, fruits, water, birds, and humans.	Bloodstream infections, cause of arthritis in individuals suffering from alcoholism	Vervaeke *et al*., 2008
Candida parapsilosis ATCC 22019 THL	Skin, hands and mucous membranes of healthy people	Emerging major human pathogen.Cause of hospital-acquired blood infections	Weems, 1992
Candida sorbosa[ana] (*Issatchekia_occidentalis* [teleo] #) *CBS 1910 CBS*	Soil (dust), clinical specimens	Food spoilage	Arroyo-López *et al.,* 2012
Penicillium chrysogenum DSM 895 HED	Moist/damp indoor environments, soil, plants Salted food, seeds, dairy barns	Important human allergens	Bancerz *et al.,* 2005

3.5 CONCLUSION

From the current study, the prevalence of various bioaerosols at the dairy farm plant was established. Indoor concentrations of airborne microorganisms were generally higher than those outdoors. Studies have reported that sources of high indoor microbial loads included shedding of human-associated microbiota (from skin, hair, nostrils and the oral cavity), oral and respiratory fluid emitted via talking, coughing, sneezing and breathing (Nicas *et al.,* 2005; Johnson and Morawska, 2009; Xie *et al.,* 2009; Fox *et al.,* 2010). The recorded microbial counts were lower than the counts indicated by most proposed standards, although this should not be considered to be the general state of most food processing/handling environments. Lack of a relationship between microbial counts and the investigated environmental parameters suggested a need for further investigations to ascertain the influence that these parameters may have on the prevalence of bioaerosols in the dairy farm plant and in food environments in general.

The fingerprinting of unknown airborne culturable microbiota using MALDI-TOF MS is a simple and rapid automated technique to identify microorganisms that is suitable for a wide variety of microorganisms (in food and environmental samples) including bacteria, yeasts and fungi. The results presented in this paper identified strains of commonly known food spoilage organisms, including pathogenic microorganisms, and suggest a need for a review and improvement of health and hygiene practices which should be maintained at all times in order to minimise the risk of potential contamination of dairy

products from airborne microorganisms. Most of the isolated microbiota were associated with soil, agricultural activities (animals and crops) and normal human flora. Furthermore, the presence of pathogenic strains that are commonly associated with hospital environments came as a concern and therefore suggest a need for further investigations in order to establish their relationship with the dairy farm environment.

The results of this research work further proved the need for agreed indoor air standards for food environments generally both locally and internationally in order to ensure proper hygiene conditions, to reduce emission of bioaerosols and also to reduce possible airborne contamination of the food and beverage products produced. In conclusion, the ability of MALDI-TOF MS to fingerprint simply and rapidly the culturable airborne microbiota was proven beyond any reasonable doubt in this study and as a result, it was concluded that MALDI-TOF MS could play a vital role in the generation of bioaerosol data which can be used towards the establishment of agreed sampling and analysis methods, as well as standards and/or limits globally.

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CHAPTER 4

DISTRIBUTION OF MICROBIAL CONTAMINANTS ON WORKING SURFACES IN THE DAIRY FARM PLANT

DISTRIBUTION OF MICROBIAL CONTAMINANTS ON WORKING SURFACES IN THE DAIRY FARM PLANT

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4.1 ABSTRACT

The colonisation of food contact surfaces in the dairy industry by microbes is a major problem as it affects the microbial safety and quality of dairy products. The purpose of this study was to assess the hygiene status of the food contact surfaces and equipment in the fresh processing plant at a dairy farm plant in central South Africa. Microbial samples were collected through swabbing and standard microbiological methods, after which quantification and fingerprinting were done. Collected swabs were diluted, cultured, quantified and matrix-assisted laser desorption ionization time of flight (MALDI-TOF MS) was used for the microbial fingerprinting. Microbial counts on the food contact surfaces ranged between 2.5 x 10^2 cfu.cm^{-2} and 1.1 x 10^5 cfu.cm^{-2} over the entire duration of the study. The most predominant strains isolated from the surfaces included food spoilers and pathogens from a genus such as *Pseudomonas, Staphylococcus, Candida, Acinetobacter, Bacillus, Rhodotorula, Aeromonas*, *Lactobacillus*, *Enterobacter, Escherichia, Klebsiella and Kocuria.* Some of these organisms were reported to have an ability to form and live in biofilm communities. The positive identification of strains from the aforementioned community of biofilms on food contact surfaces highlighted the rapidity and sensitivity of MALDI-TOF MS in the dairy processing environment which may be useful in ensuring the production of safe and high quality dairy products. The results of this study suggest that there is a fairly high probability of milk and milk products being contaminated from food contact surfaces. It is crucial therefore to improve the efficiency of sanitation, food processing and handling practices during production.

Keywords: MALDI-TOF MS, dairy farm plant, microbial communities, surface swabs**.**

4.2 INTRODUCTION

Food safety is critical for the improvement of public health through reduction and prevention of foodborne illnesses, as well as for the reduction of economic losses (Cahill, 2005). In recent years, the microbiological safety and quality of food has emerged as an important concern globally (Sofos, 2008; Nørrung and Buncic, 2008; Velusamy *et al.,* 2010). There are a number of different factors that may contribute to the contamination and recontamination of the products in the food processing environments as well as to disease manifestation and/or occurrence. Such factors may include environmental factors, host factors, and the pathogenicity of the infectious agent. The hygiene status of the processing environment, the processes undertaken and the processes and raw materials used by the food handlers, are highly significant factors for the microbiological safety and good quality of food products.

Indoor environments provide an opportunity for exposure and contamination of food by microorganisms which are highly opportunistic in that they take advantage of any favourable environment to multiply (Kowalski and Bahnfleth, 1998). People carry large numbers of microorganisms on themselves and as a result, their movement around the processing area could result to contamination of the food contact surfaces, and ultimately of the processed food products (Rahkio and Korkeala, 1997). On the farm, potential sources of surface contamination may include dust, contaminated water, food handlers, the hygiene state of the processing environment and the presence of animals in the vicinity of the processing environment (Lehto *et al.,* 2011).

In food processing environments an abundance of areas which permit attachment and proliferation of unwanted microorganisms are present. Poor hygiene measures such as inadequately cleaned food processing surfaces and equipment are a potential source of contamination which may possibly lead to the proliferation of unwanted spoilage and pathogenic microorganisms. Surfaces of food processing environments have long been recognised as microbial contamination and recontamination sources where the build-up of biofilms is prevalent (Zottola and Sasahara, 1994; Lehto *et al.,* 2011). To ensure microbiological surface control in the food processing environments, surfaces must be hygienically designed and adequate hygiene procedures must be implemented (Verran *et al.,* 2008).

The examination of the microbial communities (biofilms) on food contact surfaces is done by examining surface swabs. This is however difficult to do inside the technological equipment in the dairy plants (Chmielewski and Frank, 2003; Verran *et al.,* 2008; Schlegelova *et al.,* 2010). Adherence of microorganisms to food contact surfaces and their proliferation on equipment often results in contamination of the product, shortening its shelf-life and making it potentially microbiologically unsafe for consumption by altering its chemical composition. The aim of this study was therefore to investigate the prevalence of microbial populations on milk contact surfaces and equipment in a dairy farm plant, as well as fingerprinting using MALDI-TOF MS. This will constitute a first report using MALDI-TOF MS for surface contamination in a dairy farm setting, thereby determining its sensitivity level in identifying microorganisms from

a community of biofilms. This study will shed light in the field of food industry especially towards ensuing wholesome food and beverages.

4.3 MATERIALS AND METHODS

4.3.1 Sampling Site

The study was conducted on a 6000-hectare dairy farm that is situated in Free State province, in central South Africa. This dairy farm employs approximately 300 employees in different sections on the farm. Operations on this farm include livestock farming and crop farming (for feed for over 2000 cattles) activities with the processing of fresh dairy products also done on the same premises. A floor diagram of the said farm is attached in Appendix A (Figure A1).

4.3.2 Sampling protocol

Surface swabs were taken in order to monitor the microbial biota on the processing surfaces as well as on equipment in various processing sections of the dairy farm plant. Swabs were used because most areas are not easily accessible using Rodac plates and some areas were irregular. A total of 140 surface samples were collected over the duration of the study from a surface area of 2 x 2 cm square area. Samples were collected comprising surface swabs which were taken from processing surfaces and equipment such as 250 ml cream holder, 250 ml cream sealer, 2 litre stage, 2 litre platform, 2 litre nozzle, 2 litre capper, 3 litre stage, 3 litre platform, 3 litre nozzle, and 3

litre capper as shown in Tables 4.2 to 4.10. Samples were taken in 7 consecutive sampling cycles after sanitation instead of 10 as indicated in the previous chapter. The reason for this was because on three of the sampling days there was some unforeseen work that had to be done on some sections of the farm, hence a reduced number of samples. The same sampling times and frequency were employed throughout the sampling period.

4.3.3 Microbiological sampling and analysis

4.3.3.1 Microbiological sampling through surface swabs

Samples were taken on the aforementioned surfaces using sterile cotton swabs in 5 ml of peptone water. The samples were kept on ice during transportation to the laboratory, and processed without delay (Bryan *et al.,* 1997). Upon arrival at the laboratory, swabs were diluted to 10^{-3} and samples spread-plated on Plate Count Agar (PCA) (Merck, SA) and Potato Dextrose Agar (PDA) (Merck, SA) for the quantification of total viable count and total viable fungi respectively. Subsequent incubation of the plates was done in an inverted position at temperatures between 25ºC and 35ºC for periods that ranged from 24 to 72 hours respectively for the selected media (Rajasekar and Balasubramanian, 2011). After the desired period of incubation, the colonies formed were counted and expressed as colony-forming units per square centimetre prior to their fingerprinting using MALDI-TOF MS.

4.3.3.2 MALDI-TOF MS Analysis

Taxonomic identification and/or fingerprinting of isolated microorganisms was done by MALDI-TOF MS (Bruker Daltonics, South Africa), which provides protein profiles from each isolate. The Bruker Daltonics methodology was employed. Briefly, cells (single colonies) from biological material were recovered by scraping the plate and transferred into an Eppendorf tube with 300 µL of Ultrapur water (Merck, South Africa). This was then mixed thoroughly. Absolute ethanol (900 µL) (Merck, South Africa) was added carefully, mixed thoroughly, and centrifuged at maximum speed (1320 rpm) for 2 minutes at room temperature. The supernatant was decanted and the pellets air-dried at room temperature. The dry pellets were mixed thoroughly by vortexing with 50 µL formic acid (70%) (Merck, SA), followed by the addition of 50 µL pure acetonitrile (Merck, SA) and mixed thoroughly again. The mixture was centrifuged at maximum speed (1320 rpm) for 2 minutes, and approximately 1 µL of the supernatant was placed onto a Micro Scout Plate (MSP) 96 polished steel target plate (Bruker Daltronics, Germany) and allowed to dry at room temperature. Subsequently, each sample was overlaid with 1 µL of the HCCA matrix solution (a saturated solution of a-cyano-4-hydroxy-cinnamic acid (Sigma, USA) in 50% acetonitrile-2.5% trifluoroacetic acid) (Bruker Daltronics, Germany) and air dried at room temperature. The analysis of all strains was performed by means of a Microflex LT mass spectrometer (Bruker Daltonics, Germany) using Flex-Control software (version 3.0, Bruker Daltonics, Germany). The spectra were recorded in the linear positive mode (with the laser frequency of 20 Hz; ion source of 1 voltage, 20kV; ion source of 2 voltage, 18.6 kV; lens voltage, 7.5 kV; mass range, 2000 to 20 000 Da). For each spectrum, 240 shots in 40-

shots from different positions of the BTS spot (manual mode) were collected and analysed. The spectra were internally calibrated by using *Escherichia coli* ribosomal proteins as the standard. The raw spectra were imported into the Bio Typer software (version 3.0, Bruker Daltonics, Germany), processed by standard pattern matching with standard settings, and the results reported in a ranking table with colour codes. Outcomes of the pattern-matching process were expressed as proposed by MALDI-TOF biotyper manufacturer with identification scores ranging from 0 to 3. Scores lower than 1.70 were considered not to have generated a reliable identification; a score of between 1.70 and 1.90 was considered to have correctly identified the isolated sample to genus level and a score greater than 1.90 was used for reliable identification of the sample to species level.

4.4 RESULTS AND DISCUSSION

4.4.1 Microbial counts in surface swabs

Food contact surfaces play a major role in controlling the spread of foodborne pathogens in food processing facilities. Microorganisms on food contact surfaces are sometimes a principal cause of food contamination, potentially resulting in the spoilage of food products, transmission of foodborne pathogens and foodborne outbreaks. Table 4.1 summarises the prevalence of microbial colonies from the above mentioned swabbed surfaces. Surface swabs are usually done in order to express the degree of contamination of a particular foodstuff as well as to indicate the presence of pathogens in the food processing environment. Bacterial counts over the entire duration of the

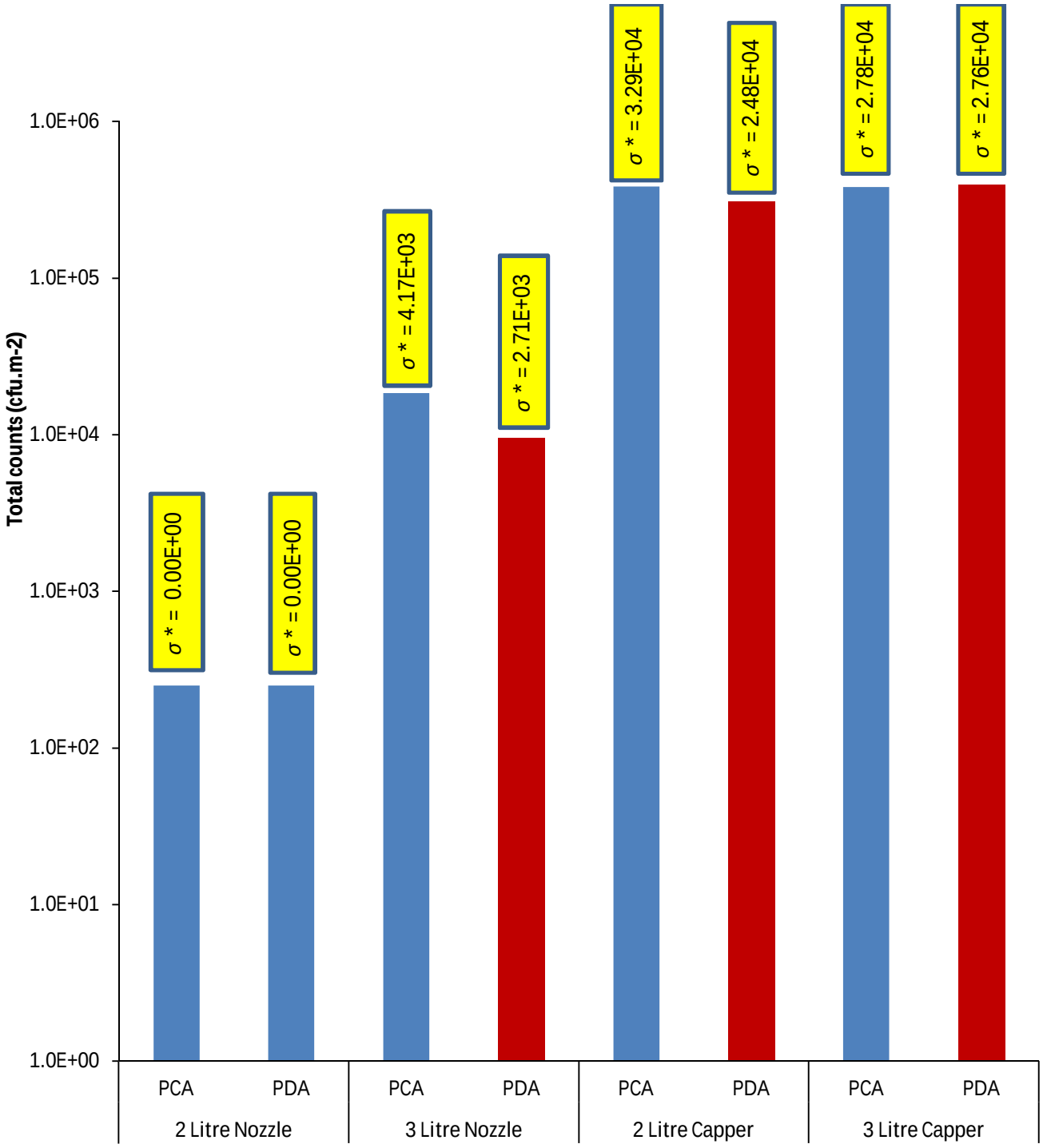
study ranged between 2.5 x 10^2 cfu.m^{-2} and 1.1 x 10^5 cfu.m^{-2}, whilst the fungal counts ranged between 2.5 x 10^2 cfu.m^{-2} and 8.6 x 10^4 cfu.m^{-2}. The lowest bacterial counts were found on the 250 ml cream sealer and three-litre stage surfaces, whilst the highest bacterial counts were found on the two-litre platform surfaces. The lowest fungal counts were found on the 250 ml cream sealer surface and the the three-litre stage whilst the highest fungal counts were observed from the three-litre capper surfaces. Frequent growth was observed from both the two- and three-litre capper surfaces, with minimal growth observed from the two-litre nozzle, two-litre stage, and the 250 ml cream sealer surfaces. The contamination and prevalence of microorganisms on food contact surfaces plays a significant role in the transmission of foodborne diseases (Rodrick, 2007).

Figure 4.1 presents the comparison of microbial loads between the two- and three-litre filler nozzle surfaces as well as between the two- and three-litre capper surfaces over the entire duration of the study. The three-litre nozzle counts (both bacterial and fungal) were generally higher in comparison with the two-litre nozzle where no microbial loads were observed. The three-litre nozzle bacterial counts were 7.4 x 10^4 cfu.m^{-2} and 3.8 x 10^4 cfu.m^{-2} for the fungal counts. Microbial loads were observed from both the two- and three-litre capper surfaces. The bacterial load from the two-litre capper surfaces was slightly higher at 3.84 x 10^5 cfu.m^{-2} in comparison with the three-litre capper surface load which was 3.80 x 10^5 cfu.m^{-2}. The fungal load from the three-litre capper surface was higher (3.9 x 10^5 cfu.m^{-2}) in comparison with the two-litre capper surfaces load which had counts of 3.2 x 10^5 cfu.m^{-2}.

Table 4.1: Microbial loads on contact surfaces in the dairy farm plant

SURFACE AREA	MEDIA	COUNTS PER SAMPLE (cfu.m^{-2})$^{\$}$							
		#1	#2	#3	#4	#5	#6	#7	σ*
2-litre nozzle	PCA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
	PDA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
2-litre platform	PCA	6.3×10^4	2.5×10^2	2.5×10^2	5.9×10^4	2.5×10^2	8.5×10^4	1.1×10^5	4.2×10^4
	PDA	6.3×10^4	2.5×10^2	2.5×10^2	3.4×10^4	2.5×10^2	7.8×10^4	4.9×10^4	3.0×10^4
2-litre stage	PCA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
	PDA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
2-litre capper	PCA	8.0×10^4	5.3×10^4	2.5×10^2	8.9×10^4	2.5×10^4	4.0×10^4	9.6×10^4	3.3×10^4
	PDA	6.8×10^4	1.8×10^4	2.5×10^2	3.8×10^4	6.0×10^4	5.1×10^4	7.2×10^4	2.5×10^4
250ml cream holder	PCA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
	PDA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
250 ml cream sealer	PCA	8.7×10^4	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	3.0×10^4
	PDA	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	0
3-litre nozzle	PCA	1.2×10^4	2.5×10^2	1.0×10^3	2.5×10^2	2.5×10^2	2.5×10^2	5×10^3	4.2×10^3
	PDA	8.0×10^3	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.7×10^3
3-litre platform	PCA	6.5×10^4	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.3×10^3	7.5×10^4	3.1×10^4
	PDA	7.3×10^4	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	4.5×10^3	2.5×10^4
3-litre stage	PCA	5.8×10^4	5.0×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	8.3×10^4	3.2×10^4
	PDA	6.4×10^4	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2	5.0×10^4	2.6×10^4
3-litre capper	PCA	7.8×10^4	6.5×10^4	3.3×10^4	2.5×10^2	8.8×10^4	4.9×10^4	6.8×10^4	2.8×10^4
	PDA	7.4×10^4	4.4×10^4	4.3×10^4	2.5×10^2	8.0×10^4	6.6×10^4	8.6×10^4	2.8×10^4

**Standard Deviation (σ), $^{\$}$All values are in scientific format to one decimal*



**Standard Deviation (σ)*

Figure 4.1: Microbial load comparison between the two- and three-litre surfaces at the dairy farm plant

4.4.2 Isolated microorganisms

In processing environments, the contamination and recontamination of food contact surfaces and equipment after cleaning and sanitisation could occur from various sources such as changes in food production processes, bioaerosols (distribution systems and ventilation systems), water, cleaning activities, drainage blockages, and waste (Verran *et al.,* 2008). The variety of possible sources of contamination found in food processing environments could favour the accumulation of microbial communities on food contact surfaces (Bower *et al.,* 1996; Gunduz and Tuncel, 2006). In the dairy industry, contamination of milk and related products commonly occurs as a result of improper cleaning and disinfection of the food contact surfaces and equipment (Gibson *et al.,* 1999; Jessen and Lammert, 2003). Due to high density food handlers a variety of microorganisms may be transported into the food processing area from the outdoors potentially resulting in the contamination of food contact surfaces which may end up being resistant to cleaning agents and survive on surfaces for prolonged periods (Radmore, 1986; Meklin, 2002).

A total of 29 genera of microorganisms were isolated from the food contact surfaces and processing equipment in the fresh processing area at the dairy farm plant using MALDI-TOF MS (Tables 4.3-4.10). About 93% of the isolated colonies were bacteria with the remaining 6.9% being fungal genera. Fifty-three (53) different species were positively identified and of these species, 92.5% were identified as bacterial species and 7.5% as fungal species. There were fifty-six (56) positively identified strains amongst

these species. The most dominant species isolated were *Pseudomonas* sp. (22.6%), *Staphylococcus* sp. (11.3%), *Acinetobacter* sp. (5.7%), *Candida* sp. (5.7%), *Bacillus* sp. (5.7%), *Lactobacillus* sp. (3.8%), and *Enterobacter* sp. (3.8%), together representing 58.6% of all the isolated species. The remaining 41.4% of all the isolated species was made up by microbial genera such as *Rhodotorula*, *Aeromonas*, *Citrobacter*, *Microbacterium, Chryseobacterium*, *Corynebacterium*, *Escherichia, Kocuria, Sphingobium, Hafnia, Herbaspirillum, Wautersiella,* to mention but a few.

4.4.2.1 Gram-positive bacterial isolates

The *Staphylococcus* genus is ubiquitously distributed in nature, as staphylococci are known to be the normal flora on the skin and mucous membrane of mammals. However, staphylococci have been isolated from a variety of foodstuffs such as meat and dairy products, as well as from environmental sources which include, amongst many others, soil, dust, sand, water and air (Kloos and Schleifer, 1986). Various strains of *Staphylococcus* are recognised for the role they play in desirable reactions such as the production of flavour and aroma reactions in fermenting foods such as dairy (i.e. cheeses) and meat (i.e. sausages) products (Irlinger *et al.,* 1997; Blaiotta *et al.,* 2004).

Table 4.2: Sample area: two-litre capper

Isolated species	Source	Implications	Reference
Acinetobacter johnsonii DSM 6963T HAM	Human skin and mucous membrane, faecal matter, soil (dust) and waste water	Vascular catheter-related bloodstream nosocomial infections	Bouvet and Grimont, 1986
Burkholderia tropica DSM 15359 HAM	Crops	Causes diseases in humans, animals and plants	Reis *et al.,* 2004
Buttiauxella noackiae DSM 9401T HAM	Surface and drinking water, soils (dust), samples from human and snail intestinal tract, raw milk and cheese	Human diseases	Muller *et al.,* 1996
Candida pararugosa 33 PIM	Human faecal matter Clinical specimen (saliva of a sarcoma patient)	Cause of infections, colonisations and persistent environmental contamination events in immune-compromised patients	Giammanco *et al.,* 2004
Chryseobacterium scophthalmum LMG 13028T HAM	Gills of diseased turbot	Pathogenic in fish Defects in dairy products	Mudarris *et al.,* 1994
Corynebacterium accolens 87_D5_coll ISB	Soil, water, plants, food products, mucosa and normal skin flora of humans and animals	A rare human pathogen	Neubauer *et al.,* 1991
Enterobacter cloacae 20105_2 CHB	Human skin and plants as well as in soil, water, sewage, intestinal tracts of humans and animals, and some dairy products	Opportunistic human pathogens	Hormaeche and Edwards, 1960
Microbacterium liquefaciens HKI 11374 HKJ	Dairy products	Human infections	Takeuchi and Hatano, 1998
Pseudomonas lundensis DSM 6252T HAM	Meat, fish, dairy products	Food spoilage	Molin *et al.,* 1986
Pseudomonas thivervalensis DSM 13194T HAM	Soil (dust)	Plant pathogen	Achouak *et al.,* 2000

Table 4.3: Sample area: two-litre platform

Isolated species	Source	Implications	Reference
Acinetobacter bouvetii DSM 14964T DSM	Soil (dust), clinical specimens, faecal matter	Nosocomial infections	Carr *et al.,* 2003
Acinetobacter johnsonii DSM 6963T HAM	Human skin and mucous membrane, faecal matter, soil (dust) and waste water	Vascular catheter-related bloodstream nosocomial infections	Bouvet and Grimont, 1986
Arthrobacter sp DSM 20125_DSM	Widely distributed in nature Hospital environments	Food spoilage, nosocomial infections	Trofa *et al.,* 2008
Bacillus safensis CIP 109412 CIP	Spacecraft and assembly facility surfaces	Not reported	Satomi *et al.,* 2006
Candida parapsilosis 26 PSB	Domestic animals, insect, soil (dust)	Septicaemia in immune-compromised patients Nosocomial infections	Weems Jr, 1992
Chryseobacterium scophthalmum LMG 13028T HAM	Gills of diseased turbot	Pathogenic in fish	Mudarris *et al.,* 1994
Enterobacter amnigenus DSM 4486T DSM	Isolated from tap water, ground water and soil	Cause opportunistic bacterial infection in man	Izard *et al.,* 1981
Hafnia alvei M110266 LDW	Isolated from various mammals, fish, birds, soil, water and a number of foods	Recognised cause of a number of illnesses, including pneumonia, meningitis, abscesses and septicaemia Food spoilage potential	Moller, 1954
Klebsiella pneumoniae ssp pneumoniae 9295_1 CHB	Normal flora of the mouth, skin, and intestines	Opportunistic pathogens in nosocomial infections	Sabota *et al.,* 1998
Pseudomonas cedrina ssp cedrina DSM 105541T HAM	Spring water, phyllosphere of grasses	Not reported	Dabboussi *et al.,* 1999
Pseudomonas cichorii DSM 50259T HAM	Water, vegetables, seeds	Food spoilage	Young *et al.,* 1996
Pseudomonas extremorientalis DSM 15824T HAM	Drinking water reservoir, soil (dust)	Not reported	Ivanova *et al.,* 2002
Pseudomonas fragi DSM 3456T HAM	Milk, meat, cheese	Food spoilage	Skerman *et al.,* 1980
Pseudomonas graminis DSM 11363T HAM	Phyllosphere of grasses	Not reported	Behrendt *et al.,* 1999
Pseudomonas koreensis LMG 21318T HAM	Agricultural environments (soil (dust))	Not reported	Kwon *et al.,* 2003
Pseudomonas proteolytica DSM 15321T HAM	Water	Not reported	Reddy *et al.,* 2004
Pseudomonas rhodesiae DSM 14020T HAM	Natural mineral water, soil (dust), coal	Not reported	Coroler *et al.,* 1997
Pseudomonas tolaasii LMG 2342T HAM	Soil (dust), crops	Major agricultural problem	Young *et al.,* 1996
Rhodotorula mucilaginosa DSM 70825 DSM	Soil (dust), water, humans (skin, respiratory, gastro-intestinal tracts) and air	Recalcitrant pathogen in immune compromised patients	Mori *et al.,* 2011
Sphingobium herbicidovorans DSM 11019T HAM	Soil (dust)	Degrade chemicals	Takeuchi *et al.,* 2001
Staphylococcus cohnii DSM 20260T DSM	Normal flora of human skin, raw milk	Rare opportunistic pathogen causing diseases in human	Schleifer and Kloos, 1975

Table 4.4: Sample area: 250ml cream sealer

Isolated species	Source	Implications	Reference
Herbaspirillum huttiense DSM 10281T HAM	Well water, agricultural soils	Plant pathogen	Ding and Yokota, 2004
Massilia timonae VA_23089_03 17 UKE	Clinical specimens	Human diseases	Lindquist *et al.,* 2003
Novosphingobium aromaticivorans DSM 12444T HAM	Soil, water, and coastal plain sediments	Emerging disease causative agents Causative agents or trigger of primary biliary cirrhosis	Takeuchi *et al.,* 2001
Ralstonia pickettii 21323_1 CHB	Moist environments such as soils, river and lakes	Opportunistic pathogen in people with weak immune systems	Yabuuchi *et al.,* 1995
Staphylococcus lugdunensis DSM 4805 DSM	Normal flora of human skin	Causes diseases in humans	Freney *et al.,* 1988

Table 4.5: Sample area: two-litre stage

Isolated species	Source	Implications	Reference
Aeromonas veronii CECT 4199T DSM	Soil (dust), animals, and water systems	Diarrhoea, wound infections, septicaemia in immune-compromised people	Martinez-Murcia *et al.,* 1992
Wautersiella falsenii 02_08_TR IBS	Human clinical isolates	Not reported	Kämpfer *et al.,* 2006

A variety of staphylococci strains that were isolated from this study were commonly found in the environment as an integral part of the natural flora (Irlinger, 2008). The isolated *Staphylococcus* strains included *Staphylococcus saprophyticus ssp bovis DSM 18669T DSM, Staphylococcus cohnii DSM 20260T DSM, Staphylococcus lugdunensis DSM 4805 DSM, Staphylococcus epidermis 10547 CHB, Staphylococcus pasteuri DSM 10657 DSM,* and *Staphylococcus simulans DSM 20324 DSM;* all of which their presence in food has never been reported to result in the spoilage; rather reported for their ability of causing infections (Tables 4.2, 4.3, 4.5, 4.7, 4.8 and 4.9).

The abovementioned *Staphylococcus* strains are classified as coagulase-negative. Coagulase-negative staphylococci strains are known not to have any food poisoning potential as there has never been a reported case of food poisoning outbreak following consumption of contaminated dairy products; however, these species are regarded as opportunistic pathogens in immune-compromised individuals as they may result in infections (Irlinger, 2008).

Bacillus species are a group of Gram-positive, aerobic spore-forming bacillus that are commonly widely distributed in nature. They are a common contaminant in a variety of foodstuffs (raw and unprocessed) and have previously been implicated in causing foodborne illnesses in human. The *Bacillus* genus also includes pathogenic species such as *Bacillus anthracis* and *Bacillus cereus.* The majority of *Bacillus* food poisoning outbreaks have been associated with the consumption of cooked food which was not

cooled properly and/or incorrectly stored, thereby providing conditions that allow microbial proliferation. These pathogenic species have previously been implicated in biofilms due to their ability to withstand harsh environments because they form spores. The strains of *Bacillus* species isolated this study are listed in Tables 4.3 and 4.10. *Bacillus* species are spore-formers which can survive heat treatments and therefore can lead to spoilage of dairy products.

4.4.2.2 Gram-negative bacterial isolates

Acinetobacter is a Gram-negative coccobacillus that has emerged as an organism of much interest in recent times as a result of its potential to cause nosocomial infections to immuno-compromised individuals worldwide and also because of its ability to quickly develop resistance to antibiotics (Van Looveren *et al.,* 2004; Hanlon, 2005). The occurrence of *Acinetobacter* in food processing environments is well documented (Bagge-Ravn *et al.,* 2003; Lagsrud *et al.,* 2006). Although *Acinetobacter* species have not been associated with foodborne disease outbreaks, they do have a record of causing public health concern, as their presence in food is an indicator of spoilage (Gennari *et al.,* 1992). From the current study, *Acinetobacter* species were the third most prolific species isolated from the food contact surfaces at the dairy farm plant. The isolated strains (Tables 4.3, 4.4, 4.5, and 4.10) of *Acinetobacter* were mainly from species that are known to be significant nosocomial pathogens that are commonly associated with increasing incidence of hospital-acquired infections (Bergogne-Bérézin and Towner, 1996).

Table 4.6: Sample area: 250ml cream holder

Isolated species	Source	Implications	Reference
Acinetobacter bouvetii DSM 14964T DSM	Soil (dust), clinical specimens	Nosocomial infections	Carr *et al.,* 2003
Acinetobacter calcoaceticus B388 UFL	Soil (dust), water sources and faecal matter	Fatal pneumonia	Bouvet and Grimont, 1986
Lactobacillus pantheris DSM 15945T DSM	Animal faecal matter	Not reported	Liu and Dong, 2002
Paenibacillus thiaminolyticus DSM 5712 DSM	Soil, water, animal. human faecal matter, clinical specimens, animals	Diseases in human and animals	Ouyang *et al.,* 2008
Pseudomonas fragi DSM 3456T HAM	Milk, meat, cheese	Food spoilage	Skerman *et al.,* 1980
Pseudomonas lundensis DSM 6252T HAM	Meat, fish, dairy products	Food spoilage	Molin *et al.,* 1986
Staphylococcus saprophyticus ssp bovis DSM 18669T DSM	Associated with domestic animals; carcasses of dead animals	Urinary tract infections	Raz *et al.,* 2005

Escherichia coli (*E. coli*) is a bacterium found in the intestinal track of humans and is indicative of faecal contamination of water as well as food products. Apart from the presence of *E. coli* on the 3-litre machine food contact surface, *E. coli* was rarely found on the food contact surfaces in the dairy farm plant. The rarity of *E. coli* on the food contact surfaces was in agreement with the general findings by Schlegelova *et al.,* (2010) when they also found low levels of *E. coli* on the indoor food contact surfaces on dairy farms. The presence of *E. coli* strains on the 3-litre capper machine surface on the dairy farm indicates post-sanitation or post-process contamination with organisms of faecal origin often caused by lack of hand hygiene on the part of the food handler (Campos *et al.,* 2009) (Table 4.9). Although the majority of *E. coli* strains are deemed not to be harmful commensals, various strains have been said to be pathogenic to humans and animals, resulting in enteric and diarrhoeal diseases as well as urinary tract infections, septicaemia and meningitis (Holko *et al.,* 2006). *E. coli* strains have previously been isolated in raw milk and dairy products in a number of outbreaks and as a result they have become a major concern in the dairy and food industry at large, having been found to survive cleaning and disinfection (Austin and Bergeron, 1995; Greyling, 1998).

Pseudomonas species play a highly critical role in the food industry, where spoilage of a variety of food products such as meat, poultry, fish and milk occurs even under low temperature conditions (Barrett *et al.,* 1986). *Pseudomonas* spp. are aerobic, Gram-negative soil bacteria that are common food spoilage organisms as they are the most frequently isolated bacteria from surfaces in the food industry (Forsythe, 2000; Simões

et al., 2008). The contamination of dairy products with *Pseudomonas* spp. can result in the reduction of the shelf-life of dairy products (Dogan and Boor, 2003). A variety of *Pseudomonas* strains were isolated from the food contact surfaces at the dairy farm plant (Tables 4.3, 4.4, 4.5, 4.8, 4.9 and 4.10). *Pseudomonas* species such as *P. fragi*, *P. lundensis and P. flourescenes* are currently the predominant Gram-negative microorganisms limiting the shelf-life of ultra heat treatment (UHT) processed milk at a temperature of 4°C (De Jonghe *et al.,* 2011). On food contact surfaces, microbial communities of *Pseudomonas* have the ability to attract and shelter other spoilage and pathogenic microorganisms (Marchand *et al.*, 2012) by forming biofilms.

Klebsiella is a Gram-negative bacterium that is commonly associated with nosocomial infections in immune-compromised people (Podschun and Ullmann, 1998). The bacterium is highly ubiquitous in nature and is known to be a part of the normal flora of the human gastro-intestinal tract, where they can be passed in faecal matter. A variety of *Klebsiella pneumoniae* strains with pathogenic potential may occur from the environment (Munoz *et al.,* 2007). On dairy farms, it is believed that wood products are the main source of *Klebsiella* (Munoz *et al.*, 2006). The *Klebsiella pneumonia* strain was isolated from the food contact surfaces at the dairy farm plant (Table 4.3). *Klebsiella pneumoniae* is an opportunistic organism that can cause mastitis in dairy cows, potentially impacting the quality of milk (Hogan and Smith, 2003).

Table 4.7: Sample area: three-litre platform

Isolated species	Source	Implications	Reference
Acinetobacter bouvetii DSM 14964T DSM	Soil (dust), clinical specimens	Nosocomial infections	Carr *et al.,* 2003
Bacillus safensis CIP 109412 CIP	Spacecraft and assembly facility surfaces	Not reported	Satomi *et al.,* 2006
Bacillus subtilis ssp subtilis DSM 5660 DSM	Soil (dust), plant, water, faecal matter, fermented food products	Supports plant growth, restores healthy bacterial communities in the body enhancing one's immune system	Earl *et al.,* 2008
Candida_lusitaniae[ana] (Clavispora_lusitaniae[teleo]) CBS 4413T CBS	Clinical specimens	Opportunistic human pathogen	Lachance *et al.,* 2003
Candida parapsilosis ATCC 22019 THL *Candida parapsilosis DSM 4237 DSM*	Domestic animals, insect, soil (dust)	Septicaemia in immune-compromised patients Nosocomial infections	Trofa *et al.,* 2008
Citrobacter freundii 22054_1 CHB	Widely distributed on plants and in soil, water and the intestines of humans and animals	Increasingly important pathogen in food Potential to colonise humans	Skerman *et al.,*1980
Lactobacillus ruminis DSM 20404 DSM	Human faecal matter, dominant bacterium in the large intestine, caecum and rectum of the healthy pig	Not reported	Sharpe *et al.,* 1973
Pseudomonas fragi DSM 3456T HAM	Milk, meat, cheese	Food spoilage	Skerman *et al.,*1980
Pseudomonas lundensis DSM 6252T HAM	Refrigerated meat	Food spoilage	Molin *et al.,* 1986
Rhodotorula mucilaginosa VML *Rhodotorula mucilaginosa DSM 70825 DSM*	Soil (dust), water, humans (skin, respiratory, gastrointestinal tracts) and air	Recalcitrant pathogen in immune-compromised patients	Mori *et al.,* 2011

Table 4.8: Sample area: three-litre capper

Isolated species	Source	Implications	Reference
Aeromonas veronii CECT 4199T DSM	Soil (dust), animals, water systems	Diarrhoea, wound infections, septicaemia in immune-compromised people	Martinez-Murcia *et al.,* 1992
Escherichia coli ESBL_EA_RSS_1528T CHB	Intestines of warm blooded organisms	Food poisoning, food product recalls, foodborne illnesses	Martinez-Murcia *et al.,* 1999
Pseudomonas extremorientalis DSM 15824T HAM	Drinking water reservoir, soil (dust)	Not reported	Ivanova *et al.,* 2002
Rhodotorula mucilaginosa VML	Soil (dust), water, humans (skin, respiratory, gastrointestinal tracts) and air	Recalcitrant pathogen in immune-compromised patients	Mori *et al.,* 2011
Staphylococcus simulans DSM 20324 DSM	Skin and urine samples of both humans and animals	Human and animal pathogen	Kloos and Schleifer, 1975

4.4.2.3 Fungal isolates

Yeasts are commercially significant in the food industry mainly because of their ability to cause spoilage of food products as well as for their desirable fermentation abilities. Yeasts are usually part of a normal daily food intake and are rarely associated with foodborne outbreaks and infections as they are used mostly in the fermentation of food and beverage products (Fleet, 2006). Yeast have an ability to grow under conditions that may be unfavourable to the growth of bacteria; they also have an ability to cause microbiological spoilage of a wide range of chilled and ambient stable products including milk and milk products (Seiler and Busse, 1990; Betts *et al.,* 1999). Yeasts are responsible for the spoilage of a wide variety of food, and various yeast species such as those from the *Candida* and *Rhodotorula* genera are known to cause human infections.

Candida, as an example from the fingerprinted strains, is a type of yeast that is generally part of the normal flora of skin, intestinal tract, mouth, rectum and vagina, although its presence in the body does not cause problems unless it becomes too prolific. *Candida* has previously been implicated in the spoilage of dairy products and other food products (Fitzgerald *et al.,* 2004). Strains from well known opportunistic *Candida* species such as *Candida pararugosa, Candida parapsilosis,* and *Candida lusitanae* were isolated from the food contact surfaces at the dairy farm plant (Tables 4.3, 4.4, 4.8 and 4.10).

Rhodotorula is a type of yeast commonly found in the components of the environment such as soil, air, ocean and lake water, and dairy products (Dworecka-Kaszak and Kizerwetter-Świda, 2011). *Rhodotorula* strains isolated from the current study were mainly from *Rhodotorula mucilaginosa* species which is known to have spoilage abilities in dairy products as well as being an opportunistic pathogen that affects mostly immune-compromised people (Tables 4.3, 4.9 and 4.10) (Frölich-Wyder, 2003).

Table 4.9: Sample area: three-litre stage

Isolated species	Source	Implications	Reference
Candida parapsilosis ATCC 22019 THL	Domestic animals, insect, soil (dust)	Septicaemia in immune-compromised patients Nosocomial infections	Trofa *et al.,* 2008
Kocuria rhizophila DSM 11926T DSM	Soil (dust), mammalian skin, fermented foods, clinical specimens, fresh water source and marine sediments	Opportunistic pathogen in immune-compromised patients causing meningitis, pneumonia and septic arthritis	Takarada *et al.*, 2008
Morganella morganii ssp sibonii Mb19277_2 CHB	Found in faecal matter of humans, animals and other mammals, normal flora of intestinal tracts in human, mammals and reptiles	Diseases in humans	Jensen *et al.,* 1992
Providencia rettgeri CCM 4504 CCM	Water Clinical specimens	Associated with diarrhoea and nosocomial infections in humans; cholera in chickens	Skerman *et al.,* 1980
Pseudomonas trivialis DSM 14937T HAM	Eggs, milk and various foods	Food spoilage	Behrendt *et al.,* 2003
Staphylococcus saprophyticus ssp bovis DSM 18669T DSM	Skin, genito-urinary mucosa, clinical specimens and animals	Opportunistic pathogen associated with urinary tract infections and the leading cause of cystisis in women	Skerman *et al.,* 1980

Table 4.10: Sample area: three-litre nozzle

Isolated species	Source	Implications	Reference
Aeromonas veronii CECT 4199T DSM	Soil (dust), animals, water systems	Diarrhoea, wound infections, septicaemia in immune-compromised people	Martinez-Murcia *et al.,* 1992
Staphylococcus epidermidis 10547 CHB	Normal flora of human skin	Nosocomial pathogen in immune-compromised individuals	Wieser and Busse, 2000
Staphylococcus pasteuri DSM 10657 DSM	Human, animal and food specimens	An emerging agent of nosocomial infections and a blood derivatives contaminant. Resistant to several antibiotics	Chesneau *et al.,* 1993

4.5 CONCLUSION

Foodborne illnesses can be controlled by implementing good health and hygiene measures in order to prevent contamination and cross-contamination of microorganisms between foods and food contact surfaces. Moisture and the availability of water, which is a necessity in the dairy processing area, are very important factors which may have contributed to the prevalence, proliferation and build-up of microbial communities on the food contact surfaces thus leading to biofilm formation. Cool water can condense on surfaces and damage them, promoting the growth of microorganisms which ultimately contaminate food and beverages and can even affect the health and well-being of employees or other occupants of the premises (IPMVP, 2002). Some microorganisms can survive and multiply even when conditions are harsh (Kristjansson and Hreggvidsson, 1995; Schöenheit and Schäefer, 1995; Stetter, 1995; Parry, 2005).

The ability of many microorganisms to adhere to surfaces and to form biofilms has been observed in a variety of environments including the food processing environments, where biofilms have major implications because they create a persistent source of contamination.

Microbial contamination of food contact surfaces have been reported to have the potential to cause food spoilage and outbreaks which may result in significant economic losses. The results of the present study showed high total microbial counts from food

contact surfaces which may be a consequence of the low level of hygiene maintained during the processing and production of dairy products. Food contact surfaces at the dairy farm plant constituted an environment that was conducive to the survival and growth of microbial communities such as *Pseudomonas, Staphylococcus, Candida, E. coli, Enterobacter, Rhodotorula, Bacillus, Acinetobacter, Corynebacterium Klebsiella, Aeromonas, Citrobacter, Hafnia, Burkholderia* and *Microbacterium*. The soil environment is known to be extensively complex and diverse, being a rich reservoir for a highly diverse microbiota, which was evidenced by the findings of this study (Adams and Moss, 2008). The presence of these spoilage microbes and pathogens on the food contact surfaces poses a serious threat to immune-compromised individuals. Proper procedures must be put in place and must be enforced to curb possible contamination during production.

The Centre for Disease Control identified poor personal hygiene as a contributing factor in some foodborne outbreaks and Rahkio and Korkeala (1997) further indicate that, because people naturally carry a lot of microorganisms, possible contamination sources within the dairy plant are increased. Although microbial strains from a variety of food spoilage microorganisms were isolated from the food contact surfaces at the dairy farm plant, a variety of strains from pathogenic microorganisms was also isolated which suggests a need for further investigation in terms of establishing the role that these pathogenic microorganisms play in the dairy processing plant.

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CHAPTER 5

The evaluaTion of food Hygiene Knowledge, Attitudes And PrActices of Food Handlers in a dairy FarmPlant in Central South AfricA

THE EVALUATION OF FOOD HYGIENE KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS IN A DAIRY FARM PLANT IN CENTRAL SOUTH AFRICA

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5.1 ABSTRACT

The purpose of this study was to assess the knowledge, attitudes, beliefs and practices (KABP) of food handlers on all levels of seniority. Hygiene aspects and production practices in the processing area of a dairy farm plant in central South Africa were also assessed. Questionnaires for the evaluation of employees concerning food safety in the dairy farm plant were developed, and data was collected from randomly selected food handlers (*n*=30) in the different processing sections in the plant through face to face interviews. Half (50%) of the respondents had been working at the dairy farm plant for less than a year. Of the 30 participants, the majority (60%) had undergone basic food safety training. The employees (40%) who had not been trained on basic food safety were mainly new employees. All respondents (100%) agreed that it was important to wash hands frequently when handling food, but had different views regarding who was responsible for food safety: 63.33% stated that it was the processors' responsibility, with 36.67% acknowledging that it was everyone's responsibility. The KABP of food handlers as well as hygienic production practices are important in ensuring a downward trend in the occurrence of foodborne illness. Results of the survey highlighted the fact that there is a need to establish and implement awareness programmes and refresher courses pertaining to food safety and general hygiene for employees as soon as they are employed, with on-going new development programmes on food safety aspects, particularly for food handlers.

Key words: dairy farm plant, hygiene**,** knowledge, attitudes, beliefs, perceptions, practices

5.2 INTRODUCTION

Food is critical for the health and well-being of consumers (Rozin *et al.,* 1999; Mutlu, 2011), and quality control is essential in the food industry for ensuring food that is safe, visually acceptable and pleasing, palatable, and consistent with food product specifications (Wilcock *et al.,* 2004; Loveless *et al.,* 2010). In the food industry, a food safety system is usually designed to prevent food safety hazards from causing foodborne disease outbreaks or illnesses; and the hazard analysis critical control point (HACCP) system is commonly used for this purpose (Educational Foundation, 2004; Green, 2008).

Food spoilage is still a moderately poorly understood process with many different aspects. It is said to be an economically significant problem for manufacturers, retailers and consumers (Zeuthen and Bøgh-Sørensen, 2003). Foodborne pathogens (disease-causing microorganisms) pose a great threat to food safety as they spoil food by degrading its quality and/or shelf-life, resulting in foodborne illnesses which affect millions of people annually (Mead *et al.,* 1999). Foodborne diseases are a common concern to the public worldwide and in South Africa, as they appear to be poorly investigated and generally under-reported (National Institute of Communicable Diseases, 2010; Niehaus *et al.,* 2011). This could be attributed to the fact that in South Africa for example, there is no appropriate infrastructure in place for the reporting of such cases to trigger investigation and also due to sporadic occurrence of these

outbreaks which often go unnoticed. The World Health Organization (WHO) (2008) reports that poor investigation of many foodborne disease outbreaks can be attributed to lack of skills or because field investigators are expected to master all skills single-handedly without having been provided with proper training.

Food contamination can occur at different stages in the food processing chain. Inappropriate and unhygienic handling of food plays a crucial role in the occurrence and spread of foodborne diseases (Republic of South Africa: National Department of Health, 2000; Baş *et al.,* 2006; Egan *et al.,* 2007). Consumption of food contaminated with foodborne pathogens or their toxins is the leading cause of foodborne illnesses in developing countries resulting with approximately 1.8 million deaths annually (Education Foundation, 2004; Jin *et al.,* 2009). A study conducted in the USA suggests that improper food handling practices contributed to approximately 97% of foodborne illnesses (Howes *et al.,* 1996; Egan *et al.,* 2007). This was later supported by a study conducted by Baş and co-workers (2006) as well as WHO (2003), who all support the notion that foodborne diseases can be spread by cross-contamination from hands that are not properly cleaned.

Most foodborne illnesses can be prevented if food safety principles are understood and practised thoroughly throughout all phases in the food chain (Jacob *et al.,* 2010). In food safety, it is of great significance to understand the interaction between knowledge, attitude, behaviour and practices of food handlers to be able to minimise the risk of food

contamination and foodborne disease or illness outbreaks (WHO, 2000). This paper presents data on a questionnaire survey that assessed the hygiene knowledge, adherence and behaviour of food handlers from a dairy farm plant in central South Africa. The study was conducted through face-to-face interviews and used a questionnaire with a series of open and closed-ended questions.

5.3 MATERIALS AND METHODS

5.3.1 Study location

This survey was conducted on a dairy farm outside Bloemfontein in central South Africa during August 2011 to assess the status of food hygiene and food safety issues including practices. The dairy farm where the survey was conducted had farming activities (i.e. livestock and crop farming) and the processing of dairy products was also done onsite (Appendix A: Figure A1).

5.3.2 Questionnaire design

A questionnaire (Appendix C) with open and closed-ended questions was administered to 30 employees from two sections (i.e. UHT and fresh sections) in the dairy farm processing plant, representing 29.7% of all food handlers. The questionnaire consisted of five sections, namely: a) employees demographics; b) food safety knowledge; c) food safety adhrence; d) health and hygiene practices; e) health and safety in the workplace.

The questions focused on matters such as knowledge of employees, attitude, knowledge, beliefs and practices in terms of hygiene aspects and production practices.

5.3.3 Data collection

Arrangements were made with the company where the study was done prior to the interviews, in order to secure consent for the gathering of information through verbal interview session and to collect product samples. Interviews were conducted by the researcher and fellow postgraduate (Master's level) students from the Unit for Applied Food Science and Biotechnology of the Central University of Technology, Free State. All these students are well trained and qualified as Environmental Health Practitioners under the Health Professions Council of South Africa. Interviewers were briefed by the researcher on how to conduct interviews and how to make objective observations regarding food safety in general. The random sampling method was used to select employees in two different plants (namely, the fresh plant and the UHT plant) at the dairy farm. Thirty (30) food handlers comprising 29.7% of all food handlers were randomly selected from different sections in the dairy farm plant. The purpose of the interviews was explained to both the superiors (section managers) and food handlers; and a special effort was made to ensure that the respondents understood the purpose of the study as well as the questions asked.

The average completion time for the questionnaire was 10 minutes. Prior to assessment, the questions were also translated into the local languages, specifically Afrikaans, Sesotho and Setswana, for people who did not understand English.

5.3.4 Data analysis

Scores for demographic information, food safety knowledge, attitude, health and hygiene practices as well as health and safety were calculated by the researcher based on the multiple choice answer to each statement, and mean responses and percentages in each category were calculated and presented in a tabular form using Microsoft Office 2010 and/or Excel 2010 for statistical purposes where necessary.

5.4. RESULTS AND DISCUSSION

5.4.1 Profile of interviewees

Table 5.1 reflects the demographic data of the food handlers (respondents) that were involved in the study. Of the 30 respondents in the study, 15 (50%) of them were female and 15 (50%) were male. Their ages ranged between 19 and 57 years, with all employees (100%) being of African descent and employed on a permanent basis at the dairy farm plant. 50% of all the respondents had been employed at the dairy farm for less than a year. Although the respondents had not achieved a notable level of education, the majority of them (86.67%) had some form of further educational training (FET) education (grade 9-12). More than 63.33% of respondents did not have any post-matric training and only 36.67% had some sort of additional training which was generally not related to food safety.

Table 5.1: Demographic data of food handlers *(n=30)*

Variable	Demographic characteristics	Response (%)
1. Gender	Male	15 (50%)
	Female	15 (50%)
2. Race	African	30 (100%)
	Asian	0 (0%)
	Coloured	0 (0%)
	White	0 (0%)
3. Age	Below 20	1 (3.33%)
	20-30	18 (60%)
	31-40	10 (33.33%)
	41 and above	1 (3.33%)
4. Language preferred	English	9 (30%)
	Tswana	11 (36%)
	Sotho	18 (60%)
	Other	5 (16.7%)
5. Employment status	Permanent	30 (100%)
	Volunteer	0 (0%)
	Other	0 (0%)
6. Level of education	None	0 (0%)
	Grade R-8	4 (13.33%)
	Grade 9-12	26 (86. 67%)
	Tertiary Education	0 (0%)
7. Working experience	Below 1 year	15 (50%)
	1-2 years	3 (10%)
	2-3 years	5 (16.67%)
	3-4 years	2 (6.67%)
	More than years	5 (16.67%)
8. Additional training?	Yes	11 (36.67%)
	No	19 (63.33%)

5.4.2 Knowledge of food handlers regarding food safety and hygiene

Internationally, the World Health Organization (WHO) has identified food safety as one of its top ten priorities (WHO, 2008). The safety of food is of critical importance to the food industry, the consumer (in terms of health and well-being) and the economy of the country (Jevšnik *et al.,* 2008). The scores indicating food handlers' knowledge are presented in Table 5.2. Respondents had different views when it came to who was responsible for food safety: the majority (63.33%) stated it was entirely the food producer's/processor's responsibility, with the remaining 36.67% reporting that it was everyone's responsibility. It was noted that the 36.67% of respondents who acknowledged that food safety was everyone's responsibility were from the group of food handlers (50%) who had less than one year's work experience at the dairy farm plant.

The respondents had different views when it came to the question of why food safety was important, with the majority (80%) of food handlers reporting that food safety was mainly important for the prevention of illnesses; 60% indicated it was important to make food fit for human consumption, and 43.33% stated that it was important for the preservation of food. This resulted in the responses for the question totalling over 100%. The 50% of new employees accounted for the 40% of employees not trained in food safety. These scores were consistent with those obtained from the study done by Baş *et al.* (2006); they also reported that the majority (47.8%) of food handlers had not undergone food safety training. Data from a study done by Buccheri and co-workers (2007) also revealed that 78.1 to 87.7% of food handlers had never attended any

training or course on food safety (food hygiene and foodborne diseases) which may suggest that there is a trend of food handlers not being trained on food safety although they may be working with food. The majority (90%) of food handlers indicated that, if given an opportunity, they would attend training and/or further training about food safety and only 10% of food handlers gave a negative answer stating that they would not attend any training on food safety as they were not provided with any certificates after such training.

5.4.3 Adherence of food handlers to food safety and hygiene measures

Attitude and adherence are important factors when it comes to the reduction of foodborne diseases (Nee and Sani, 2011). Table 5.3 shows the responses in regard to the attitudes of food handlers. In a study done by Afifi and Abushelaibi (2012), it is reported that most foodborne diseases were caused by poor personal hygiene, improper handling of food and inappropriate use of temperatures. From the current survey, 90% of the respondents agreed that adherence to correct temperatures during food processing was essential to ensure food safety. All (100%) of the respondents in the current study agreed that frequent hand washing is a necessity when working with food whilst 96.66% said that keeping surfaces clean when working with food reduces the risk of food contamination, thereby preventing/avoiding illness (Table 5.3).

Table 5.2: Food handlers' responses about food safety and hygiene knowledge *(n=30)*

Statement	Answer	Response %
1. Who is responsible for food safety?	Food processor or producer	63.33%
	Consumer	0 (0%)
	Everyone (i.e. both producers and consumers)	36.67%
	Other (specify)	0 (0%)
2. In your opinion, why is food safety important?	To prevent illness	24 (80%)
	To preserve food	13 (43.33%)
	To make food fit for human consumption	18 (60%)
	It is not important	0 (0%)
	Other (specify)	0 (0%)
3. Have you had any training in food safety?	Yes	18 (60%)
	No	12 (40%)
4. Referring to question 3, which of the following did you attend?	HACCP*	2 (6.67%)
	GMP[#]	3 (10%)
	GHP[$]	18 (60%)
	Other (specify)	1 (3.33%)
4. If yes, what type of training?	Full course	0 (0%)
	Workshop(s)	20 (66.67%)
	Other (specify)	0 (0%)
5. Would you go for training/further training in food safety?	Yes	27 (90%)
	No	3 (10%)

(Some respondents had multiple answers in question 2; hence the response percentage exceeds 100% when added)

**Hazard Analysis Critical Control Point*
[#]*Good Manufacturing Practices*
[$]*Good Hygiene Practices*

To reduce the incidence of foodborne illnesses, it is necessary to improve food handling practices and food safety campaigns (Wong *et al.,* 2004). Reports from a study conducted by Clayton *et al.* (2003) indicate that unsupervised hand washing will never be compliant in any work setting however, in the current study food handlers complied with this aspect.

All (100%) of respondents agreed that the freshness and appearance of food upon delivery is important, and 86.66% of food handlers agreed that storage practices have an impact on food safety. Although 40% of food handlers were not trained in food safety, the majority of food handlers (96.67%) showed awareness of food safety by agreeing that attaining knowledge and training on food was important for food safety.

In general, from the six questions that were presented, respondents showed a good attitude towards food safety and hygiene as they mostly agreed with the questions asked. In contrast, previous reports from a study done by Baş *et al.* (2006) indicate that the attitude scores of food handlers towards prevention of foodborne diseases (44.2 ± 13.2) as well as safety practices (48.4 ± 8.8) were very low.

Table 5.3: Food handlers' responses indicating attitudes towards food safety and hygiene *(n=30)*

Statement	Response [number (%)]		
	Agree	**Disagree**	**Not sure**
1. Frequent hand-washing during and between processing is necessary	30 (100%)	0 (0%)	0 (0%)
2. Keeping surfaces clean reduces the risk of illness	29 (96.67%)	0 (0%)	1 (3.33%)
3. Adhering to correct temperatures during processing is useful to ensure food safety	27 (90%)	0 (0%)	3 (10%)
4. Storage practices have an impact on food safety	26 (86.67%)	3 (10%)	1 (3.33%)
5. The freshness and appearance of food (including milk products) upon delivery is important	30 (100%)	0 (0%)	0 (0%)
6. Knowledge and training are important in ensuring food safety	29 (96.67%)	1 (3.33%)	0 (0%)

5.4.4 Health and hygiene production practices

Food handling and preparation procedures differ significantly in different food industries according to the type of food handled, the processes followed and the food handler's knowledge in terms of food safety (Ropkins and Beck, 2000). Responses about health and hygiene are displayed in Table 5.4 (a & b). Hygiene surrounding the handling of raw materials and the processing environment is a very important factor for the microbiological safety and quality of final products (Lehto *et al.,* 2011). Table 5.4 (a) clearly shows that the majority (93.33%) of food handlers knew that there was a health and safety representative in the processing area, and 60% of the food handlers stated that they had undergone training on good health and hygiene measures.

From the results, it was also observed that the 40% of respondents who had not attended any training on good health and hygiene measures came from the group of employees who had been working at the dairy farm for less than a year. Only 6.66% of the food handlers had been trained on HACCP and both of them had been working at the dairy farm plant for more than 7 years. In contrast, Garayoa *et al.* (2011) report that 41.9% of food handlers interviewed in their study were informed and/or trained regarding HACCP. Although 40% of the respondents in the current study had not received any training on good health and hygiene, all of them (100%) concurred that it was important to wash hands before handling food, during and after working with food, as well as after using the toilet facilities. All respondents (100%) agreed there was a procedure available for washing hands.

As indicated in Table 5.4 (b), 80% of the respondents said that they cleaned the production working area and surfaces before, during and after work, with 23.33% and 16.67% stating that they clean before and after a day's work respectively. In relation to hand washing in the processing area, the majority (90%) of respondents said that they cleaned their hands before, during and after work. The respondents also indicated that they sanitised their hands after every fifteen minutes during processing. Reports in a study done by Collins (2001) indicate that lack of personal hygiene amongst food handlers was one of the most commonly reported sources of foodborne illnesses.

South African legislation clearly stipulates that no persons will be permitted to handle food if they do not wash their hands with soap and hot water (RSA, 1999). Most (73.33%) respondents reported that they used water, soap, nail brush and disposable towel to clean their hands, with the remaining respondents (26.67%) saying they only washed their hands with soap and water without drying afterwards or using the nail brush. All respondents (100%) acknowledged that there was a procedure that they used or followed at the processing plant to wash their hands. Hot water is known to be more effective when washing hands with soap. From Table 5.4 (a) it is clear that there are mixed results regarding the water that respondents used to wash hands, with more than 56.67% saying they used both hot and cold water to wash their hands, 26.67% reporting that they used mainly hot water and the remaining 16.67% reporting that they used cold water for the purpose of washing hands.

Table 5.4 (a): Respondents' health and hygiene production practices *(n=30)*

Statement	Response [number (%)]	
	Yes	**No**
1. Is there a health and safety representative in the processing area?	28 (93.33%)	2 (6.67%)
2. Have you been trained on good health and hygiene measures?	18 (60%)	12 (40%)
3. Have you been trained on food safety (HACCP)?	2 (6.67%)	28 (93.33%)
4. Is it important to wash your hands before handling food?	30 (100%)	0 (0%)
5. When do you need to wash your hands?		
• Before, during and after working	30 (100%)	0 (0%)
• After sneezing/coughing	28 (93.33%)	2 (6.67%)
• After touching your hair/face (nose, mouth)	28 (93.33%)	2 (6.67%)
• After touching waste or potentially contaminated surfaces such as rubbish bins	29 (96.67%)	1 (3.33%)
• After toilet	30 (100%)	0 (0%)

Table 5.4 (b): Respondents' health and hygiene production practices *(n=30)*

Statement	Answer	Response [number (%)]
1. How often do you wash/clean the working area/surfaces?	Before the day's work	5 (16.67%)
	Before, during and after work	24 (80%)
	After a day's work	7 (23.33%)
2. How often do you wash your hands?	Before the day's work	2 (6.67%)
	Before, during and after work	27 (90%)
	After a day's work	1 (3.33%)
3. If you do, what do you normally use?	Water	0 (0%)
	Water and soap	8 (26.67%)
	Water, soap, nail brush and disposable towel	22 (73.33%)
4. Is there a procedure for washing hands and surfaces?	Yes	30 (100%)
	No	0 (0%)
5. What water do you use to wash your hands?	Cold	5 (16.67%)
	Hot	8 (26.67%)
	Both	17 (56.67%)
6. What do you use to dry your hands?	Disposable towel	27 (90%)
	Cloth	0 (0%)
	Toilet paper	3 (10%)
	Own clothing	0 (0%)
	Hand air dryer	0 (0%)
	Nothing	0 (0%)

(Some respondents had multiple answers in question 1, hence the response percentage exceeds 100%)

5.4.5 Health and safety practices

Health and safety in the workplace is crucial so as to protect the employer and employees as well as other people who may be adversely affected by the activities taking place in and around the workplace. It is every employer's responsibility to ensure that employees' health and well-being is not compromised.

The occupational health and safety practices are represented in Table 5.5. 70% of food handlers stated that material safety data sheets (MSDS) were readily available at their workplace, 5% that there were no MSDS available, and the remaining 13.33% said that they did not know what an MSDS was. The majority (96%) of respondents said there was a lockable storage place for all chemicals used in the processing area. More than 86.67% of food handlers said there was a first aider readily available for assistance in emergency situations. The majority of food handlers (96.67%) said they reported any wounds or cuts to the first aider for dressing prior to working with food.

Table 5.5: Respondents' occupational health and safety practices *(n=30)*

Statement	Response [number (%)]	
	Yes	**No**
1. Is there a material safety data sheet file available for the processing area?	21(70%)	5 (16.67%)
2. Is there a lockable storage area for all chemicals used in the processing area?	29 (96.67%)	0 (0%)
3. Is there a first aider in the processing area?	26 (86.67%)	4 (13.33%)
4. What do you normally do if you have a wound?		
• Report it	0 (0%)	0 (0%)
• Cover it with a cloth	1 (3.33%)	0 (0%)
• Report it and apply dressing	29 (96.67%)	0 (%)
• Nothing	0 (0%)	0 (0%)

5.5. CONCLUSION

Food hygiene at dairy farm processing plants requires special attention in order to reduce the contamination risk of milk and its products. The role of food handlers in the contamination of food has been emphasised by a number of authors (Maguire *et al.,* 2000; Koopmans and Duizer, 2004). Although a number of studies have been done, the data available suggest that in order to improve food safety there is still a need to further investigate the relationship between knowledge, attitude, behaviour and practice (KABP) in order to stimulate the downward spiralling of occurrences of foodborne diseases (WHO, 2000).

Findings of this study demonstrated that the majority of food handlers who were not trained on food safety and who said that food safety is the processors' responsibility came from the group of food handlers with less than one year of work experience at the dairy farm plant. Although employees with less than a year's experience accounted for 40% of the employees who were not trained on food safety, all employees agreed that it was important to wash hands before, during and after working with food. This was a positive note, as a previous report by Collins (2001) revealed that poor hand and surface hygiene, together with poor personal hygiene of food handlers, were some of the commonly reported practices that led to foodborne disease outbreaks.

Although it is known that knowledge transferred through training courses may not necessarily result in the desired change in attitudes and behaviour (Seaman and Evans,

2006; Pilling *et al.,* 2008), food hygiene training is still important as it ensures food safety knowledge and reduces the possibility of cross-contamination that may result in foodborne outbreaks. However, provision of the necessary facilities, support and motivation from superiors may be critical in the success of food safety training which may in return contribute to the changes in knowledge, attitude, behaviour and practices that are needed (Todd *et al.,* 2007; Soon and Baines, 2012). Results of the survey highlighted the need to train employees on food safety and general hygiene as soon as they are employed, and to provide ongoing refresher programmes.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

In spite of the continuing progress made in the food industry over the past decades, the safety and quality of food products remain a critical issue worldwide, with foodborne disease outbreaks continuing to affect the health of consumers adversely, and resulting in major economic losses (Abee and Kuipers, 2011; Nada *et al.,* 2012). Contamination of food may occur at any point during transportation of raw materials, production/processing, packaging and/or distribution (Green *et al.,* 2005). The contamination of food products, transmission of pathogens and the prevention of foodborne illnesses largely depends on the food handler's personal hygiene, health status, knowledge, attitude, behaviour and his or her food hygiene and handling practices (Mead, 1999; De Bees *et al.,* 2009).

In addition to the above, contamination of food by microbial communities from the food handlers and working surfaces in both domestic and industrial environments is a common problem as the majority of foodborne illness outbreaks occur because of poor and inappropriate food handling practices (Jullien *et al.*, 2002; Vlková *et al.,* 2008; Jones and Angulo, 2006). Apart from the possible sources of contamination as mentioned, airborne microorganisms (bioaerosols) have long been acknowledged to have the potential to contaminate food in processing areas such as dairy plants (Radmore, 1986; Ren and Frank, 1992; Whyte, 2002; Salustiano *et al.*, 2003; Shale and Lues, 2007). Lack of documented literature on the distribution and proliferation of bioaerosols in various food processing environments has led to the underestimation of

their impact on the quality of food products as well as employee health and well-being (Kang and Frank, 1989; Shale and Lues, 2007). The limitation of studies on bioaerosols has also been due to the lack of agreed sampling methods, lack of agreed standards and/or limits, and relatively high cost of analysis instruments amongst other reasons (Górńy and Dutkiewicz, 2002; Douwes *et al.,* 2003; Shale and Lues, 2007).

With food being a basic need, consumers' level of interest in food safety and quality has increased immeasurably over the last decade (Nada *et al.,* 2012). Quality control and food safety issues are fundamental in the food industry, and most importantly in the dairy sector where milk, which is a very good substrate for the growth of microorganisms, is used (Wilcock *et al.,* 2004; Abee and Kuipers, 2011). It is for these reasons that it is imperative to identify and recognise the possible sources of contamination as well as contributing practices in the dairy processing plants, which may possibly lead to foodborne illnesses and economic losses (Strohbehn *et al*., 2008).

The purpose of this study was to assess microbial contaminants and related environmental parameters in a dairy farm plant in central South Africa. Chapter 3 reports on the airborne culturable microbial population both outside and inside the processing area at the dairy farm plant as well as climatic (environmental) parameters that may possibly play a role in the prevalence, proliferation and further distribution of airborne microbial populations at the dairy farm plant particularly, in the processing areas. Chapter 4 reports on the microbial populations on food contact surfaces, as this relates to the handling practices presenting a measure of the hygiene level in

processing area. In terms of the empirical work, Chapter 5 reports on the food hygiene knowledge, attitudes, and practices of food handlers, as very little work has been done in this area in dairy farm plants.

There is a wide range of well-proven analytical methods (physical, biological and chemical) and classical microbial techniques (such as microscopy and cultivation) that are used to ascertain the prevalence and characterise the composition and activities of airborne microorganisms (Martinez *et al.,* 2004; Cruz and Buttner, 2007). Physical analytical methods which are mainly based on the size and shape determination are considered to be relatively rapid, however they lack specificity (Van Wuijckhuijse *et al.,* 2005). In addition, various biological methods that are used for the detection and identification of bioaerosols are based on biological activity of microbial particles, but extensive periods may be required to perform adequate assays.

Collection of culturable microbial airborne contaminants on MALDI target plates (stainless steel) is a novel chemical analytical method that is one of the fastest ways of analysing microorganisms by mass spectrometry. This method has been used in various fields through ion detection of the molecule protein, peptide and nucleic acid of the sample, thereby detecting and fingerprinting it (Kim *et al.,* 2005). For the purpose of this study, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltronics, Germany) was used for the analysis and fingerprinting of unknown colonies in order to identify and characterise the quantified

microorganisms (Jurinke *et al.,* 2004; Van Wuijckhuijse *et al.,* 2005; Salaun *et al.,* 2010; Wolters *et al.,* 2011).

6.2 SUMMATIVE REMARKS: CHAPTER 3

Chapter 3 reports on the prevalence of airborne microbial (bioaerosol) communities at the dairy farm plant as well as the related environmental parameters that may possibly contribute to the survival and proliferation of airborne microbial contaminants. Air samples were collected through impaction on agar using a single stage (SAS Super-90) surface air sampler (PBI International, Milan, Italy), quantified, and colonies were analysed and finger-printed using matrix-assisted laser desorption ionization time of flight (MALDI-TOF MS) (Bruker Daltronics, Germany). Indoor concentrations of culturable airborne microorganisms were generally higher than those outdoors. Both microbial counts on PCA and PDA were within the ranges suggested for bioaerosol limits by a variety of agencies and authors such as Kang and Frank (1989); American Conference of Governmental Industrial Hygienists (1989); World Health Organisation (1990, 2002); and Cox and Wathes (1995) amongst others.

Environmental (climatic) parameters have been known to play a pivotal role in the prevalence, magnitude and proliferation of airborne microbes. However, this seemed not to be the case during the study. There was no difference between environmental parameters in the indoor and outdoor environments. The results presented in this chapter identified strains of commonly known food spoilage organisms, including

pathogenic microorganisms of which the majority were associated with the agricultural environment, agricultural activities (crop and livestock farming), hospital environments and normal human flora. Some of the most commonly identified culturable airborne microbiota at the dairy farm plant included amongst others *Acinetobacter* spp., *Arthrobacter* spp., *Bacillus* spp., *Candida* ssp., *E. coli* spp., *Streptococcus* ssp., *Clostridium* spp, *Staphylococcus* spp., *Aspergillus* spp., *Penicillium* spp., and *Pseudomonas* spp. The identified spoilage and pathogenic microorganisms raised a concern and suggested a dire need for strong hygienic measures as well as the improvement of mechanical ventilation systems at the dairy farm plant. With the South African historical weather records between 1974 and 2011 showing the trends of wind direction in central South Africa over the course of an average year to be from the northerly (14%), north easterly (11%), north westerly (9%), south westerly (10%), and westerly (10%) directions, it was suggested that the position of the access door at both of the processing plants should be re-evaluated.

6.3 SUMMATIVE REMARKS: CHAPTER 4

The hygiene level on food contact and preparation surfaces in the fresh processing section at the dairy farm plant were quantitatively evaluated and the microbial communities were evaluated using MALDI-TOF MS. Ten food contact surfaces were sampled and microbial loads quantified. From the results, it was evident that food contact surfaces such as the filler nozzles (i.e. two- and three-litre filler nozzles), capper machines as well as the cream holder and cream sealer surfaces had high microbial loads which could possibly lead to cross- and post-contamination of dairy products.

This suggested that the level of hygiene on the aforementioned food contact surfaces was poor and therefore a potential hazard.

Twenty-nine microbial genera comprising fifty-three species were isolated from the food contact surfaces at the dairy farm plant. The isolated species included *Pseudomonas* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Aeromonas* spp., *Bacillus* spp., *Candida* spp., *Citrobacter* spp., *Enterobacter* spp., *Lactobacillus* spp., *Rhodotorula* spp., *Microbacterium* spp., *Chryseobacterium* spp., *Corynebacterium* spp., *Escherichia* spp., *Kocuria* spp., *Hafnia* spp., *Herbaspirillum* spp., *Microbacterium* spp., *Sphingobium* spp., and *Wautersiella* spp. amongst others. From the aforementioned species, of which some are known food spoilage and pathogenic microorganisms and some have an ability to form biofilms, fifty-six microbial strains were positively fingerprinted. The strains were from a variety of sources mainly including environmental sources such as soil (dust), air, plant, water sources, and human as well as agricultural activities (such as crop and livestock farming).

The prevalence of strains from a group of microorganisms that are known to be colonisers of food contact surfaces and common food spoilers was expected. However, the isolation of pathogenic microorganisms that had not previously been isolated at food processing environments came as a surprise and led to serious concern, suggesting that there is a need for further investigation in order to ascertain the role they play at the dairy farm plant. These isolated microorganisms were rather known for the role they

play in causing diseases particularly nosocomial infections to the immune-compromised in hospital environments. Furthermore the findings of the study suggest a need for more and improved sanitation programmes.

6.4 SUMMATIVE REMARKS: CHAPTER 5

The food hygiene knowledge, attitudes and practices of food handlers at the dairy farm plant were assessed by means of a questionnaire survey. Thirty food handlers were randomly selected for the survey. The majority of the food handlers interviewed at the dairy farm plant had some form of education, although none of them had tertiary education. Half of the employees interviewed reported that they had been working at the dairy farm plant for a period of less than one year. Although 40% of food handlers had not undergone any training on good health and hygiene production practices, only 6.66% of the 60% of trained food handlers had been trained on HACCP. This was identified as a critical point which has a potential to result in the contamination and spoilage of the dairy products produced at the dairy farm plant. Overall, the results of the study revealed that food handlers had good knowledge and awareness about food safety, as well as positive attitudes towards the production of good quality and safe dairy products. The food handlers also showed satisfactory production practices as well as good health and hygiene practices at the dairy farm plant. However, a need was identified for food handlers to be trained on food safety and general hygiene as soon as they become employed at the dairy farm plant, in order to improve their knowledge and contribute to changes in attitude, behaviour and practices. Refresher training courses

need to be implemented at regular intervals so as to keep food handlers abreast of all the new developments in the dairy industry.

6.5 RECOMMENDATIONS

From the results of this study, the following points were identified as possible ways to improve food safety and quality at the dairy farm plant. These recommendations highlight possible improvements to current dairy farm plant processing methods which may also be used by other dairy farmers.

- At the dairy farm plant, possible sources of bioaerosols include livestock, crop farming, irrigation systems, manure-covered floors and walls, animals feeds (both spoiled and mould-contaminated), ventilation systems that are not working properly, water and dairy employees. All of the above should be managed and maintained in good hygienic condition in order to reduce the microbial loads in the atmosphere and the possible prevalence of bioaerosols around the dairy farm as well as in the processing area.
- Ventilation systems should be serviced regularly and maintained in good working order to effectively and adequately supply and distribute fresh air in the processing area.
- Artificial or natural barriers should be considered between kraals, feed storage area, manure storage area, crop farming area and the processing area so as to reduce odours and spread/migration of airborne microbes to other areas at the dairy farm plant.

- Considering the average climatic data around central South Africa between 1974 and 2011, the position of access doors, particularly in the fresh processing plant, should be re-evaluated so as to try and reduce the possibility of airborne contaminants being blown into the processing area as result of the wind direction.
- Employees working with cream in the fresh processing area should be monitored on a regular basis to ensure that their health is good and also to ensure that their hygiene status is satisfactory.
- Employees at the diary farm plant should be trained on food safety and general hygiene prior to resuming duties in the different sections of the dairy farm plant.
- Health and hygiene procedures as well as sanitation programmes at the dairy farm plant, particularly in the processing areas, should be reviewed as they have a potential of adversely affecting the safety and quality of the milk and milk products produced.

6.6 FUTURE RESEARCH/PROJECTS

From the results of this study, the following were identified as possible future research opportunities:

- The relationship between some of the species isolated at the dairy farm plant which are not usually associated with food, but rather associated with causing nosocomial infections in hospital environments.
- Compilation of all bioaerosol data from various studies conducted in food processing environments with the objective of compiling agreed bioaerosol limits or standards nationally and internationally.

- Compilation of a predictive bioaerosol monitoring model in dairy farm environments with the objective of controlling the magnitude of airborne microorganisms at dairy farm plants, particularly in the processing area.
- The frequent isolation of aforementioned genera whose pathogenic status in bioaerosols is yet to be clearly established in different food processing settings suggests a need for further investigations.
- Increase awareness that the quality of air in indoor food processing environments is critical to a healthy and productive work force as well as to the safety and quality of food products.

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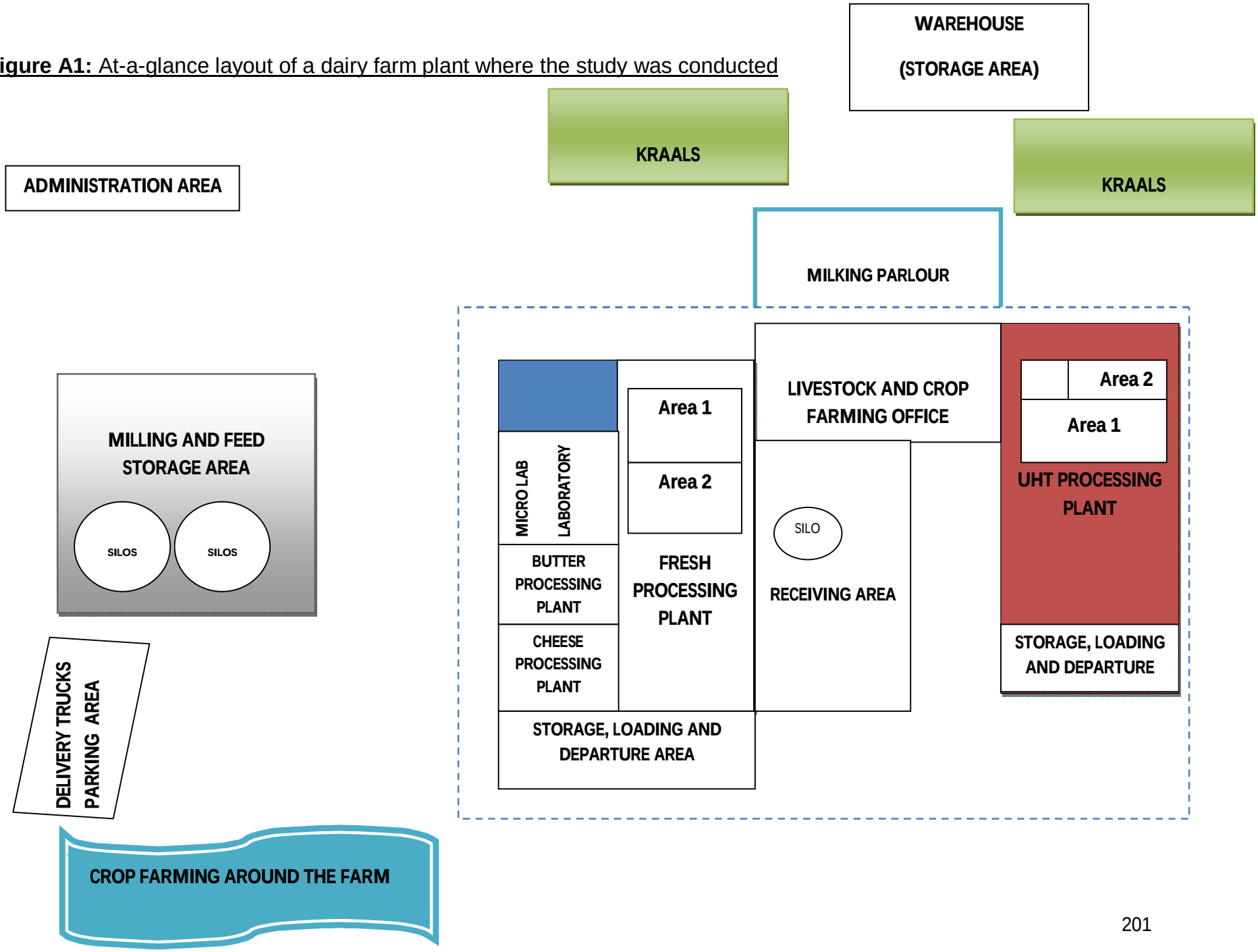
APPENDICES

APPENDIX A: AT-A-GLANCE LAYOUT OF THE DAIRY FARM PLANT

Figure A1: At-a-glance layout of a dairy farm plant where the study was conducted



APPENDIX B:

PILOT STUDY RESULTS IN A RESEARCH ARTICLE FORMAT

(Presented in poster format at the Indoor Air 2011 conference in Texas, USA)

ACCEPTED FOR PUBLICATION AS A RESEARCH NOTE IN SCIENTIFIC RESEARCH AND IMPACT ISSN 2315-5396

SELECTED BIOAEROSOLS AND EXTRINSIC FACTORS IN A DEVELOPING SEMI-URBAN DAIRY PLANT

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Abstract

Food products differ in their biochemical composition and therefore are susceptible to spoilage by different microorganisms prevalent in the atmosphere including airborne microbes. Although a number of studies have been done in different food processing settings, little is still known about the effect of airborne contaminants in the dairy industry where milk, which is an ideal substrate for the growth of microorganisms, is used. Lack of literature could possibly be attributed to lack of standards and relatively high costs of instrumental analysis although new techniques and analytical methods have been identified recently. This study focuses on indoor airborne contaminants as well as on extrinsic environmental factors influencing their distribution in a South African semi-urban dairy plant. The microbiota assessed in the air included total viable counts, total coliforms, Gram-negative, Gram-positive bacteria and fungi associated with food safety. The spread of airborne contaminants throughout various sub-sections of the dairy plant are reported in addition to the influence of temperature, relative humidity, wind speed and airborne particulates. Correlations between airborne microbes and environmental parameters are explored. It is recommended that thorough, regular monitoring of sick employees should be done, and increased ventilation and maintenance of HVAC are required. In conclusion, bioaerosol limits should be developed and more research done to understand bioaerosols better in order to be able to come with better predictive models.

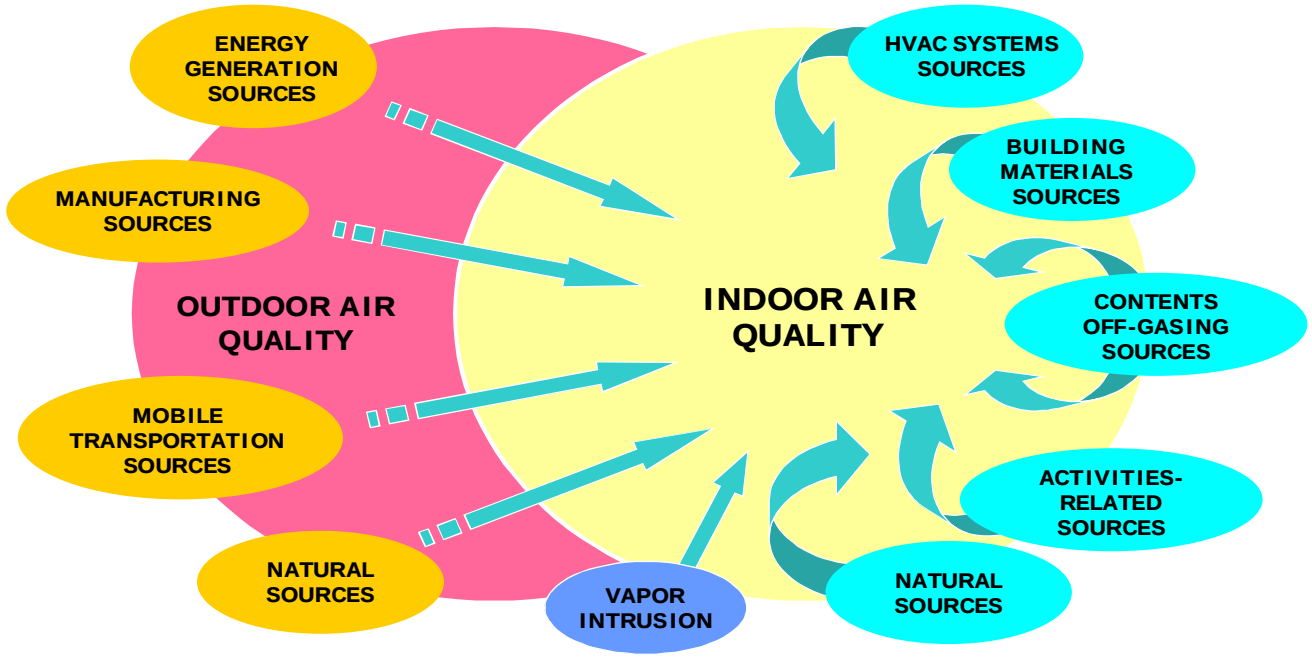
***Keywords**:* bioaerosols, food safety, dairy environments, indoor air

1. INTRODUCTION

Food spoilage is said to be an economically significant problem worldwide for manufacturers (processors), retailers, and consumers (FAO/WHO, 1999; Roller, 1999; Zeuthen and Bøgh-Sørensen, 2003); and over the last two decades, the prevalence of foodborne diseases has increased notably in both developed and developing countries (Rocourt *et al.,* 2003). In recent years, numerous incidents of foodborne disease occurrence have been reported in South Africa (Republic of South Africa: Department of Health, 2007). Research has shown that airborne contaminants can, to a certain extent, influence the quality of the food products (Shale and Lues, 2007). This opinion was also expressed by Jullien and co-workers (2002), when they reported that pathogenic microorganisms' ability to contaminate surfaces is a serious concern in the food industry.

The role of bioaerosols in various industrial settings has been well studied; however, the role of these airborne microorganisms in the South African food industry, particularly the dairy sector, is poorly understood. The quality of milk in South Africa is a matter of concern and a number of studies done so far have shown this (Greyling, 1998; O'Ferrall-Berndt, 2003; Jansen, 2003; Lues *et al.,* 2003). Kang and Frank (1989) and Salustiano *et al.* (2003) report that dairy products are more susceptible to contamination by airborne microorganisms. The trade of milk in the peri-urban, urban and rural areas has been going on for decades, however, and hygiene aspects as well as the related indoor air contaminants remain a challenge in most of the areas where milk is processed (Greathead, 1991; O'Ferrall-Berndt, 2003; Lues *et al.,* 2003; Shale and Lues, 2007).

Smaller dairy producers supply milk directly to the consumers through bulk tank milk in local shops (Jansen, 2003; Agenbag, 2008). Most of the time, this milk is of poor quality due lack of good hygiene measures (O'Farrell-Berndt, 2003). Milk from a cow is known to contain some bacteria and somatic cells, which constitute the biological constituents of milk (Turner and Veary, 1990; Gillespie *et al.,* 2009) and these milk characteristics, present a favourable environment for the multiplication of microorganisms (Gilmour and Rowe, 1981; Lues *et al*., 2003). The spoilage of milk and milk products is thus a potential hazard to human health due to contamination by emerging heat resistance pathogens, emergence of antimicrobial resistance in zoonotic pathogens, chemical adulteration of milk, and airborne contaminants as depicted in Figure 1 (Muir, 1996; Bonfoh *et al*., 2003; Ruegg, 2003; Salustiano *et al.,* 2003; Aaku *et al.,* 2004; Vasselli, 2005; Shale and Lues, 2007). The main aims of this study were to isolate and enumerate airborne microorganisms (Total Coliforms, Total Gram-positive, Total Gram-negative, Total yeast and mould) as well as to evaluate the effect of extrinsic environmental factors on the presence and multiplication of airborne microbes within semi-urban (small scale) milk processing plants.



(Lutgring *et al.*, 1997; Douwes *et al.*, 2003; Guo *et al.*, 2004; Van Tonder, 2004; Vasselli, 2005; Shale and Lues, 2007)

Figure 1: Sources of contamination showing total indoor air quality (scheme taken from Vasselli, 2005).

2. MATERIALS AND METHODS

Bioaerosol sampling

All microbial samples were collected at a height of 1,5m above the floor by means of impaction on soft agar plates. A single stage (SAS Super-90) surface air sampler (PBI International, Milan, Italy) was used for this purpose. The air sampler was calibrated at an airflow rate of 0.03 $m^3.min^{-1}$ and all the detachable parts were pre-autoclaved and disinfected with 70% ethanol between each sample run (Venter *et al.*, 2004; Shale *et al*., 2006; Coccia *et al.,* 2010). Plate Count Agar (PCA) (Merck, SA) and Potato Dextrose Agar (PDA) (Merck, SA) were used for the quantification of total aerobic count and yeast and moulds respectively. All impacted plates were incubated in an inverted position at standardised, appropriate temperatures and days (Rajasekarand Balasubramanian, 2011) with all colonies expressed as colony-forming units per cubic meter ($cfu.m^{-3}$) of air sampled.

Settling plate technique and isolation of microorganisms

For the settling plate method, the aerosolised microorganisms were collected on an open petri dish containing suitable culture media. When the sampling session was over, the petri dishes were closed and incubated at 35°C for 48 hours, 25°C for 3-5 days and for 37°C for 24 hours for aerobic plate count, yeasts and moulds, and total coliform and *S. aureus* respectively (Salustiano *et. al.,* 2003*).* For the isolation of indicator organisms *Escherichia coli*, *Salmonella*,

Staphylococcus aureus and the total viable aerobic organisms as well as the total viable fungi, Plate Count Agar (PCA), Chromocult Coliform Agar (CCA), Baird Parker (BPA) and Potato Dextrose Agar (PDA) (Merck, SA) with a pH=3.5 (tartaric acid) were used. Subsequent incubation of the plates was done at appropriate temperatures and incubation periods.

Environmental parameters

Environmental parameters, namely temperature, relative humidity and wind velocity were evaluated at all identified locations simultaneously with the sampling of microorganisms during the dry and wet seasons. These parameters were monitored during sampling which was done during an 8-hourly work shift. The evaluation was done in triplicate at a height of 1.5m above the floor (Venter *et al*., 2004). The same sampling times and frequency were employed throughout the sampling period for the different parameters of interest in this study. The following instruments were used:

- Temperature and relative humidity were measured using a heat stress monitor (QUESTemp °32; Quest Technologies Inc., Oconomowac, WI); and
- wind velocity was measured using a Vane airflow anemometer (Airflow Instrumentation LCA 6000 VT; High Wycombe, Buckinghamsire).

3. RESULTS AND DISCUSSION

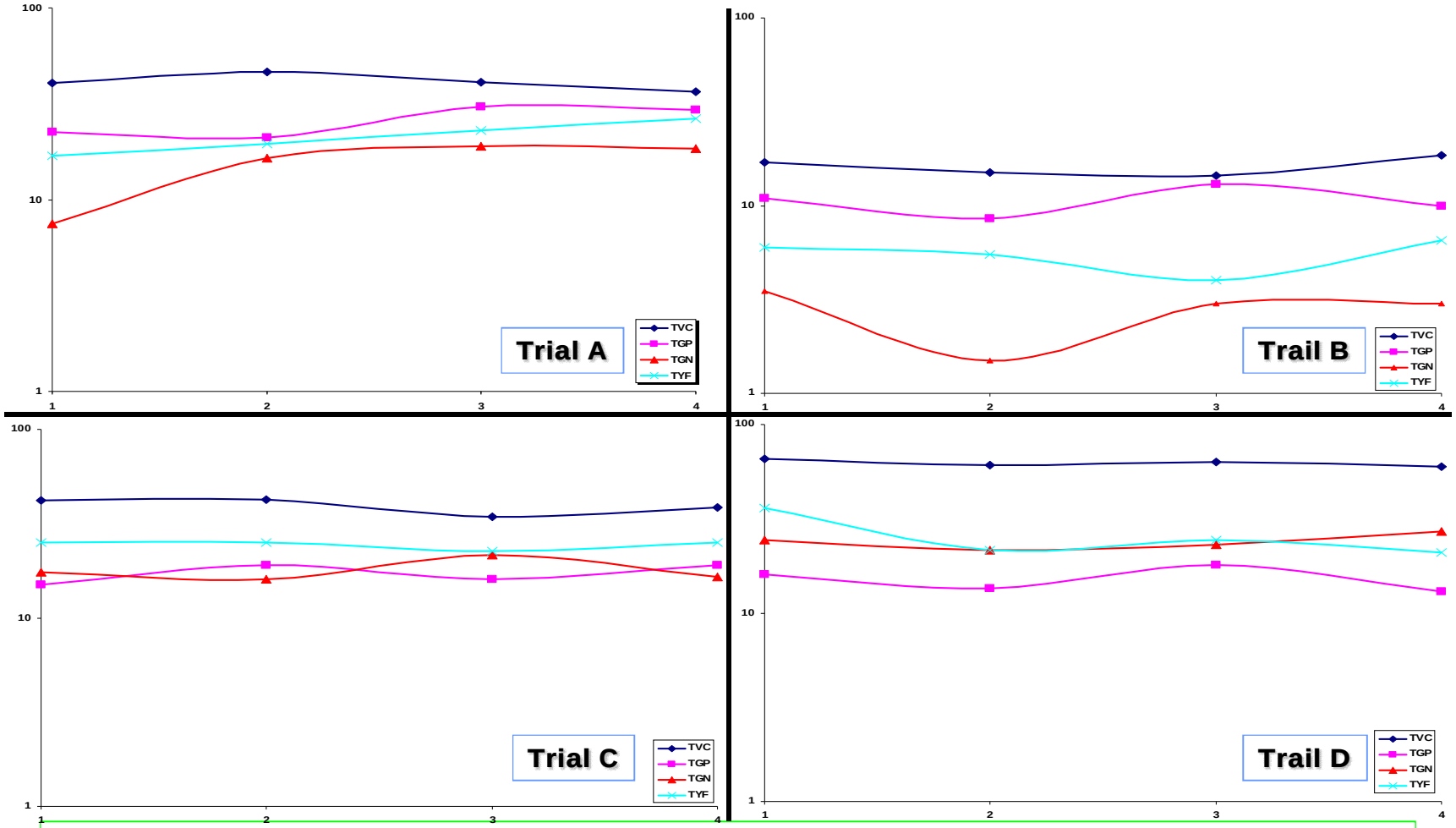


FIGURE 2: Average culturable airborne microorganisms isolated from breathable air in a small scale dairy plant. TVC (Total Viable Counts); TGP (Total Gram Positives); TGN (Total Gram Negatives) and TYF (Total Yeasts and Moulds).

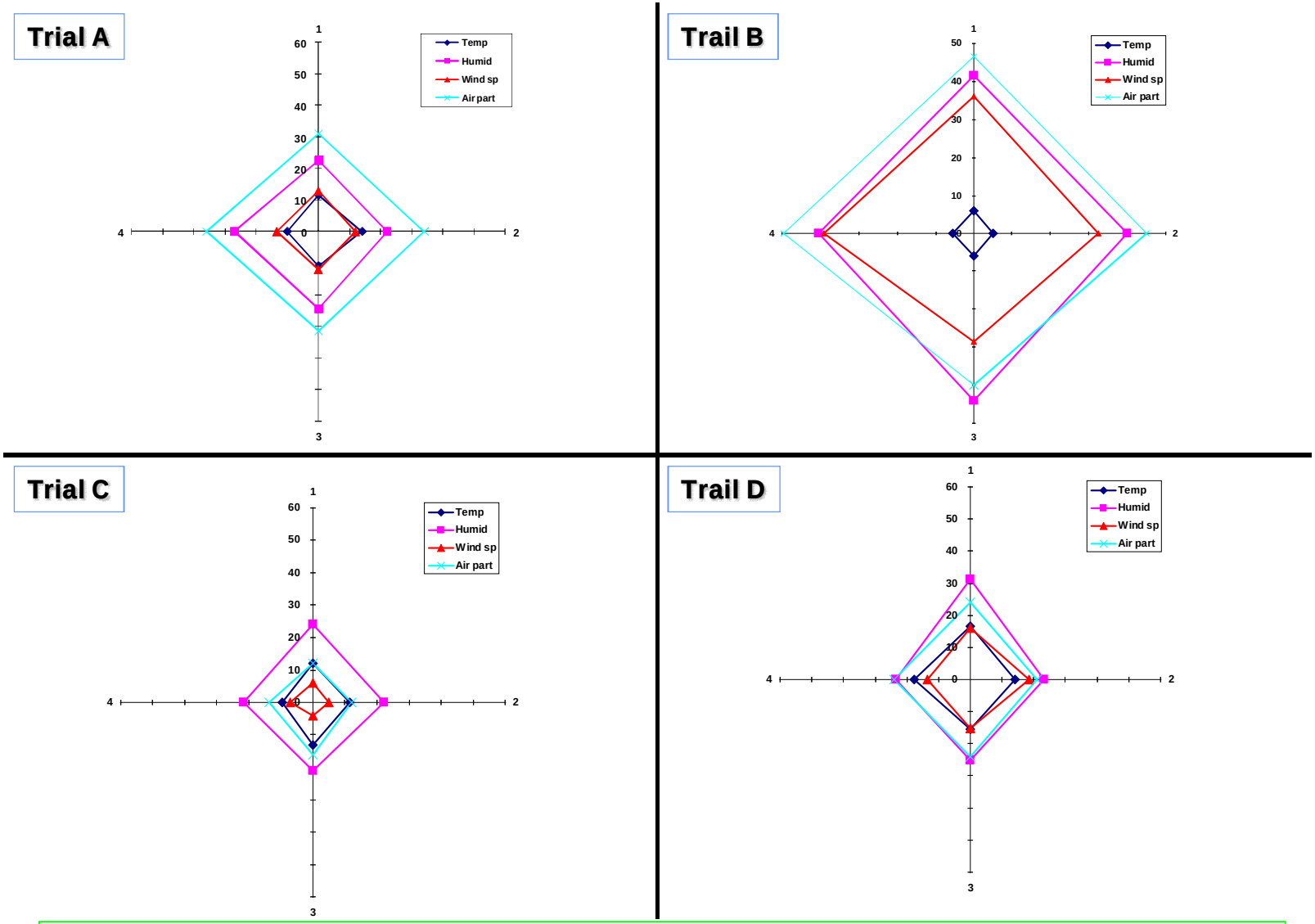


FIGURE 3: Environmental factors (Temperature ºC; Relative Humidity %; Wind velocity $m.s^{-1}$; Airborne particles $mg.m^{-3}$) quantified in a small scale dairy plant over 4 hours during various sampling trials.

According to Figure 2, bioaerosol concentrations were lower than the levels recommended by Kang and Frank (1989) for mesophilic aerobic bacteria (180-360 cfu.m^{-3}) and for yeasts and moulds (70-430 cfu.m^{-3}). These levels were also lower than the limits proposed by Ren and Frank (1992) in a milk processing plant and lower than a minimum of 100 cfu.m^{-3} accepted by the American Conference of Governmental Industrial Hygienists (1989) and the World Health Organisation (1990, 2002). The microbial numbers in the present study were below 100 cfu.m^{-3} and this suggests that most bioaerosols did not survive well in the air, thus concurring with a previous study by Salustiano *et al*. (2003). These low numbers could also be attributed to the use of non-selective media leading to stiff competition between microbes.

Figure 3 illustrates that in this project temperature affected the levels of bioaerosols, agreeing with the study by Heldman (1974) and that of Venter *et al*. (2004). Low temperatures throughout the study (Figure 3) can be attributed to the winter season when the project was conducted. Temperature levels demonstrated momentous sway on the concentration levels of airborne microbes (Figures 2 and 3), concurring with several studies (Theron, 2003; Noe, 2006; Van Tonder, 2006). Relative humidity, wind velocity and airborne particles were on average higher during trial B due to activities used by workers to warm the working area (Figure 2). High relative humidity showed no relation to bioaerosols when compared with previous studies (Venter *et al*., 2004; Manyatsa, 2007). High concentrations of total gram positives during trial A could be due to poor hygiene practices by the workers. The number of consumers coming in and out also plays a role in the variations observed in this study as airborne particles were

higher during these periods. Strong, weak positive and mostly negative correlations were noted between bioaerosols and environmental parameters.

4. CONCLUSION

- Disparities from the study can be ascribed to facility design, setup and workers' activities in the small-scale dairy plant.
- Environmental factors are not the only possible source of bioaerosol distribution in the small scale dairy plant studied.
- Lack of relation between certain environmental factors and microbial levels suggests a need for more in-depth studies on the influence of extrinsic factors on bioaerosols.
- Recorded microbial counts which are lower than most proposed standards should not lead to respite of research on indoor air contaminants in food and beverage plants.
- Good personal hygiene practices on the part of workers should be encouraged.

5. RECOMMENDATIONS

- Use of masks during milk processing could play a significant role in reducing the distribution of airborne staphylococci.
- Use of air conditioning to direct air flow to counter current production flow could also assist in less airborne contamination.
- Reduction of outdoor airborne sources gaining entry into indoor spaces is required.

- The research community must place greater emphasis upon obtaining data that correlates exposure to indoor airborne contaminants with productivity, human health implications and food quality.
- Increase recognition and awareness of workers that indoor air quality is far more dangerous to human health than is outdoor air quality.

6. FUTURE RESEARCH

Based on the outcomes of the present project, the authors plan to focus on the following aspects in beverage processing plants in South Africa. Further studies may be conducted: to review bioaerosols and related airborne contaminants in various beverage processing plants; to investigate the prevalence of related microbiota and allergens; to determine the physical and chemical parameters and their relation to indoor air contaminants; to assess airborne endotoxins and possible mycotoxins; to develop a dispersion model; and to suggest standards for the South African food and beverage processing plants in terms of bioaerosols and other airborne contaminants.

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APPENDIX C:

QUESTIONNAIRE USED FOR DATA COLLECTION IN CHAPTER 5

A SURVEY OF THE HEALTH AND HYGIENE ASPECTS AS WELL AS THE PRODUCTION PRACTICES AT A TYPICAL DAIRY FARM PLANT DURING PROCESSING IN CENTRAL SOUTH AFRICA.

Introduction

A. **All the workers in a dairy plant as well as the floor manager will be interviewed.**
B. **The answers to the questions in this questionnaire will be regarded as strictly confidential.**
C. **Mark the chosen answer with an X.**

SECTION A: THE DEMOGRAPHIC DATA

1. Date

2. Which language do you speak?

English
Tswana
Sotho
Other (specify): ……

3. Gender

Male
Female

4. Race

African
Asian
Coloured
White

5. Age

Below 20 ☐

20-30 ☐

31-40 ☐

41 and above ☐

6. Employment status

Permanent ☐

Volunteer ☐

Other (specify): ……..

7. Level of education

None ☐

Grade R-8 ☐

Grade 9-12 ☐

Tertiary education ☐

8. Additional training?

Yes ☐

No ☐

If yes, specify when: ……

9. How long have you been working at the dairy?.....

SECTION B: ADHERENCE OF INTERVIEWEE

Please indicate your opinion regarding the following by stating whether you agree or disagree:

1. ***Frequent hand-washing during and between processing is necessary***

   Agree ☐

   Disagree ☐

   Not sure ☐

2. ***Keeping surfaces clean reduces the risk of illness***

   Agree ☐

   Disagree ☐

   Not sure ☐

3. ***Adhering to correct temperatures during processing is useful to ensure food safety***

   Agree ☐

   Disagree ☐

   Not sure ☐

4. ***Storage practices have an impact on food safety***

   Agree ☐

   Disagree ☐

   Not sure ☐

5. ***The freshness and appearance of food (including milk products) upon delivery is important***

Agree ☐

Disagree ☐

Not sure ☐

6. ***Knowledge and training are important in ensuring food safety***

Agree ☐

Disagree ☐

Not sure ☐

SECTION C: KNOWLEDGE OF INTERVIEWEE

1. ***Who is responsible for food safety?***

Food processor or producer ☐

Consumer ☐

Everyone (i.e. both producers and consumers) ☐

Other (specify): ……

2. ***In your opinion, why is food safety important?***

To prevent illness ☐

To preserve food ☐

To make food fit for human consumption ☐

It is not important ☐

Other (specify): ……

3. Have you had any training in food safety?

Yes ☐

No ☐

4. Referring to question 3, which of the following did you attend?

HACCP ☐

GMP ☐

GHP ☐

Other (specify): ……

5. If yes, what type of training?

Full course ☐

Workshop ☐

Other (specify): ……

6. Would you go for training/further training in food safety?

Yes ☐

No ☐

SECTION D: HEALTH AND HYGIENE PRODUCTION PRACTICES

1. ***Is there a health and safety representative in the processing area?***

   Yes ☐

   No ☐

2. ***Have you been trained in good health and hygiene measures?***

   Yes ☐

   No ☐

   If yes, specify when: ……..

3. ***Have you been trained in food safety (HACCP)?***

   Yes ☐

   No ☐

   If yes, specify when: ……..

4. ***Is it important to wash your hands before handling food?***

   Yes ☐

   No ☐

5. ***When do you need to wash your hands?***

   Before, during and after working ☐

   After sneezing/coughing ☐

   After touching your hair/face (nose, mouth) ☐

   After touching waste or potentially contaminated surfaces such as rubbish bins ☐

After toilet

	Yes	No
Before, during and after working		
After sneezing/coughing		
After touching your hair/face(nose, mouth)		
After touching waste or potentially contaminated surfaces such as rubbish bins		
After toilet?		

6. How often do you wash/clean the working area/surfaces?

Before the day's work	
Before, during and after work	
After a day's work	

7. How often do you wash your hands?

Before the day's work	
Before, during and after work	
After a day's work	

8. If you do, what do you normally use?

Water	
Water and soap	
Water, soap, nail brush and towel	

9. Referring to question 7, is there a procedure for washing hands and working surfaces/areas?

Yes	
No	

10. Referring to question 7, what water do you use to wash your hands?

Cold	
Hot	
Both	

11. With what do you dry your hands after washing?

Disposable towel	
Cloth	
Toilet paper	
Own clothing	
Hand air dryer	
Nothing	

12. Do you mix the recent milk with the previous milk?

Yes	
No	

13. How often do you replace the tank?

Twice a week	
Once a month	
Often	

14. How often do you wash your bulk tank?

Twice a week	
Once a month	
Daily	
Other (specify):	

15. How do you wash your bulk tank?

Using chemicals and water	
Only with water	
Rinsing and scrubbing	

16. What kind of water do you use for washing the bulk tank and processing machines?

Cold	
Hot	
Both	

17. The method used when washing the tank?

By hand	
By spraying	
By brushing	
All of the above	

18. What kinds of washing chemicals are used?

Liquid soap	
Bar soap	
Disinfectants	

SECTION E: OCCUPATIONAL HEALTH AND SAFETY PRACTICES

1. Is there a Material Safety Data Sheet file available for the processing area?

Yes	
No	

2. Is there a lockable storage area for all chemicals used in the processing area?

Yes	
No	

3. Is there a first aider in the processing area?

Yes	
No	

4. What do you normally do if you have a wound?

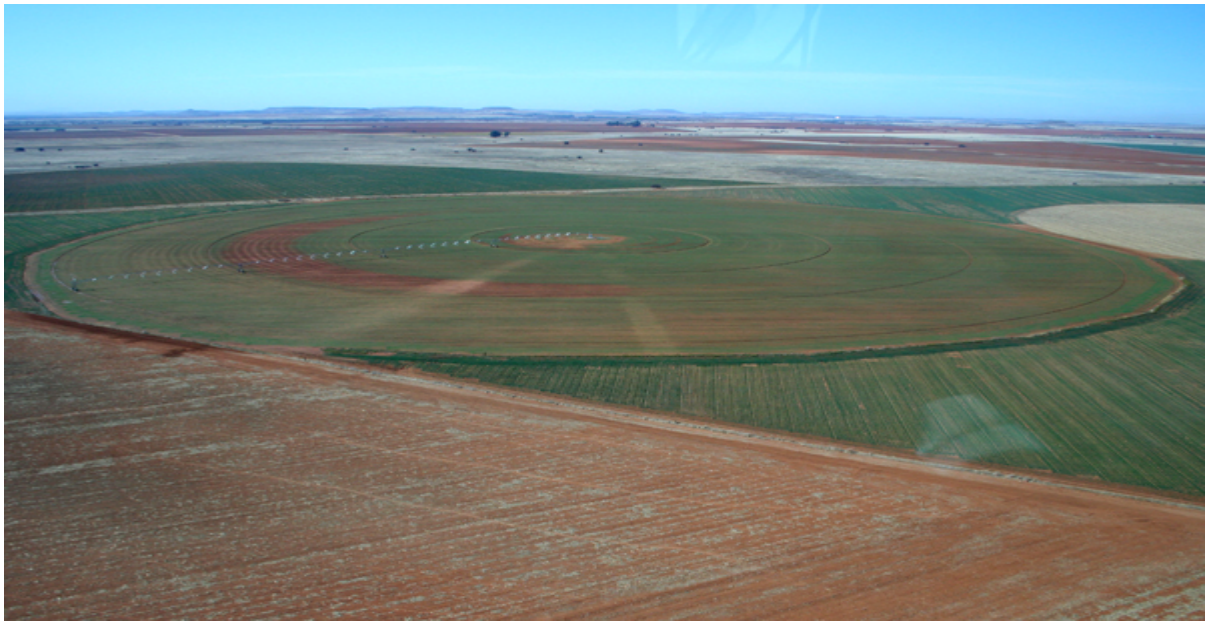
Report it	
Cover it with a cloth	
Report it and apply dressing	
Nothing	

APPENDIX D:

DAIRY FARM PICTURES

Source: Dairy farm where the study was conducted

Figure D1: Ayrshire herds in the barn area



Source: Dairy farm where the study was conducted

Figure D2: Farm area for the livestock feeds

Source: Dairy farm where the study was conducted

Figure D3: Dairy processing area

Source: Dairy farm where the study was conducted

Figure D4: Ayrshire herd