THE INFLUENCE OF EXTRINSIC AND INTRINSIC PARAMETERS ON THE QUALITY OF COTTAGE CHEESE

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Thesis submitted in fulfilment of the requirements for the degree

MAGISTER TECHNOLOGIAE:

ENVIRONMENTAL HEALTH

in the

Department of Life Sciences

at the

Central University of Technology, Free State

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Bloemfontein, South Africa, 2014

DECLARATION OF INDEPENDENT WORK

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

Modise ..... 27/11/2014 .....

SIGNATURE OF STUDENT DATE

ACKNOWLEDGEMENTS

I wish to thank the National Research Foundation (NRF) for financial support and the Central University of Technology, Free State, for the provision of facilities which contributed towards the success of this research project.

I also wish to express my sincere gratitude to the following:

- My Almighty Father, for His love, mercy and grace that kept on shining upon me until now.
- My family, my grandmother (Elisa Modise), my father (Meshack Modise), mother (Keneilwe Tebang), brothers and sisters for their countless prayers.
- Prof. Karabo Shale, my supervisor (Tshwane University of Technology, Department of Environmental Health), for his constructive and honest criticism.
- Dr Willem Groenewald for his outstanding guidance, support, encouragement, patience and belief in my abilities throughout the course of this research, and Dr Ntsoaki Malebo, for her guidance and constructive criticism.
- The Management and staff of the dairy plant that participated, especially from the cottage cheese section.
- Colleagues in the Unit of Applied Food Science and Biotechnology, most importantly: Ms Nthabiseng Nhlapo, Mr Edmore Kativu, Mrs Lebogang Shilenge and Mr Katleho Mokoena for their help, encouragement and valuable support during laboratory work.
- I would also like to express my fondest gratitude to the Medical Research Council (MRC), the South African Symposium Dairy Technology (SASDT) and Academy of Science of South Africa (ASSAF) for opportunities in presenting some chapters from this study at their conferences.

SUMMARY

Cottage cheese is a product of drained fresh cheese curd with a mild acidic flavour. The texture can be chunky or smooth; and in South Africa, cottage cheese comes in flavoured varieties that include biltong, chives, garlic and herbs. Although cottage cheese is a nutritious dairy product, it is prone to spoilage by *Pseudomonas* spp., *Staphylococcus* spp., *Listeria* spp., *Escherichia coli*, *Candida* spp. and *Debaryomyces* spp. amongst others. Spoilage of cottage cheese may occur due to several contamination of: cleanliness of equipment surfaces used during production, airborne microbes within the environment, hygiene practices of food handlers, and the quality of water used during production. Here is, however, only limited information available with regard to contamination in the production of cottage cheese in South Africa.

This study focused on the assessment of bioaerosols (airborne microbes) in a typical cottage cheese manufacturing facility, the organic acid profile changes during storage at refrigeration and room temperatures, and the knowledge, practices and behaviour of factory workers in relation to food hygiene in cottage cheese factories. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) was used to identify bacteria and fungi down to species level from equipment surfaces and bioaerosols. High Pressure Liquid Chromatography (HPLC) was further used for the identification of organic acids found in cottage cheese during shelf life. Lastly, questionnaires and an observational checklist were used for gathering data from food handlers during the study.

Bacterial counts isolated from equipment surfaces utilised during the production of cottage cheese ranged between 0.8 x 10^3 cfu.cm^{-2} and 2.8 x 10^2 cfu.cm^{-2}, with fungal

counts ranging between 0.5 x 10^2 cfu.cm^{-2} and 1.6 x 10^2 cfu.cm^{-2}. The bacterial counts from bioaerosols (from the production, packaging, shrink wrap and pasteurization areas) ranged between 72 cfu.m^{-3} and 92 cfu.m^{-3} counts with average fungal counts of 53 cfu.m^{-3} and 58.5 cfu.m^{-3}. Microbes isolated included species from genera *Staphylococcus*, *Lactobacillus*, *Bacillus, Pseudomonas*, *Candida, Micrococcus, Enterobacter* and *Acinetobacter* amongst others*. Staphylococcus aureus* in dairy products such as milk, yoghurt and cheese have been reported to cause foodborne disease outbreaks. These species were isolated from equipment, including the moulded cheese container before and after salting. *Lactobacillus* spp. are known to be associated with milk products and *Lactobacillus coryniformis*, *Lactobacillus lactis* and *Lactobacillus mali* were isolated from cheese vat 1, cheese vat 4 and the cheese scale. *Lactobacillus lactis* ssp*. cremoris* and *Lactobacillus lactis* ssp. *lactis* are mainly used in the production of cottage cheese as they have the ability to utilise milk citrate to produce characteristic flavour compounds. *Bacillus cereus* on the other hand was isolated from cheese vat 4 while *Bacillus pumilus* and *Bacillus licherniformis* were isolated from the cheese scale. The presence of *Bacillus cereus* in undercooked meat products and unpasteurized milk has been reported to cause foodborne disease outbreaks. *Bacillus licherniformis* and *Bacillus pumilus* are not human pathogens nor toxigenic, although they have been implicated in the spoilage of dairy products.

As far as it could be determined, this is the first report of the isolation of *Pseudomonas* spp., *Staphylococcus* spp., *Candida* spp., *Lactobacillus* spp. and *Acinetobacter* spp. from bioaerosols in the cottage cheese section of a dairy plant. Cottage cheese samples were stored at 4°C and at a room temperature of 27°C to determine their spoilage rates and to assess the type and quantity of organic acids within the cottage cheese. Spoilage of cottage cheese may result if improper refrigeration conditions and the wrong starter cultures are used during production stages. From the second week of sampling, test results from cottage cheeses showed that the pH of the cottage cheese sample stored at 4°C was slightly higher (pH 4.25) than that of the cottage cheese

sample stored at room temperature 27°C (pH 4.15). Spoilage of cottage cheese occurred from week 3 for cottage cheese stored at 27°C. Results obtained from HPLC on organic acids in cottage cheese samples stored at 4°C and 27°C confirmed the presence of oxalic, orotic, citric, lactic, acetic, fumaric and uric acids. Formic, acetic, butyric and propionic acids are volatile acids, contributing to the aroma of cottage cheese.

Questionnaires and an observational checklist were used to gather information from the food handlers in the cottage cheese section of a dairy plant. During the production of cottage cheese it was observed that 60% of the food handlers used their bare hands (put their hands inside cheese vats) to feel if the curd was cooked to a desired state. Cross-contamination of cheese may originate from cheese vats, cheese cloth, production room air, floor surfaces, packaging material, starter cultures, brine and curd cutting knives. Thirty percent of food handlers admitted to not cleaning the manufacturing equipment after use. Insufficient sterilization of equipment surfaces remaining damp after sanitization allows the attachment of spoilage and pathogenic organisms leading to contamination. Contamination of cottage cheese due to improper food handling practices and poor hygiene status of the cottage cheese section could lead to its spoilage. Results from this study indicate that bioaerosols, the hygiene practices of food handlers, and microbial contaminants from equipment surfaces influence the spoilage potential of cottage cheese. There is currently a lack of agreed standards on bioaerosols in the food sector worldwide. Lack of data may cause under-estimation of the spoilage potential of bioaerosols, and there is therefore a need for agreed standards worldwide.

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LIST OF ABBREVIATIONS

COA	Certificate of Acceptability
EHP	Environmental Health Practitioner
GMP	General Management Practices
HACCP	Hazard Analysis Critical Control Points
HPLC	High Performance Liquid Chromatography
HVAC	Heating, ventilation and air conditioning
MALDI TOF MS	Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry
PPE	Personal Protective Equipment
SANS	South African National Standards
SAS	Surface Air Sampler
SHE	Safety, Health and Environment
VOC	Volatile Organic Compounds
µL	micro litre
$m.s^{-1}$	meters per second
$cfu.m^{-3}$	Colony forming units per cubic meter
$cfu.g^{-1}$	Colony forming units per gram
$cfu.m^{-2}$	Colony forming units per square meter
$cfu.ml^{-1}$	Colony forming units per millilitre

mg.ml^{-1}	milligram per millilitre
ηm	nanometer
mg.kg^{-1}	milligram per kilogram
E. coli	*Escherichia coli*
Cal	Calcium
Cholest	Cholesterol
Poly	Polysaccharide
Mono	Monosaccharide
Na	Sodium
LAB	Lactic Acid Bacteria

INTRODUCTION

CHAPTER 1

1.1 BACKGROUND

In 2005, worldwide milk production was estimated at 644 million tons per annum of which South Africa contributed approximately 0.5% (Kutzemeier, 2006; Anonymous, 2007; DAFF, 2011). Table 1.1 represents the number of milk producers and milk production per province in South Africa. The majority of dairy farms are situated in the Free State Province, North West Province and Western Cape Province with smaller numbers in Gauteng and Mpumalanga Provinces (Statistics survey, 2006). Milk is sourced from cows, sheep, goats, horses, camel, buffalo and yaks (Mead *et al*., 1999; Parker, 2003) with the majority coming from cows (Jay, 2000; DSA, 2006).

The composition of milk from dairy cows is water (87 to 89%), fat (3.3 to 3.4%), protein (3.2%), lactose (4.6 to 4.8%), salts (0.6%) and enzymes such as proteases, reductases, phosphatases, lactoperoxidases, catalase and lipases amongst others (Scott, 1998; Smit *et al*., 1998; Haug *et al*., 2007). The constituents of milk play an important role in the production of dairy products. Milk is the primary ingredient of dairy products such as butter, consumer milk, condensed milk, dried milk, cheese, yoghurt and ice cream. Other typical by-products of milk include whey, buttermilk, sour milk and their derivatives (Figs 1.1 and 1.2) (Mead *et al*., 1999; DAFF, 2011). Milk is prone to microbial contamination due to a high water content, lactose, milk fat and milk proteins (Lues *et al*., 2003; Parker, 2003) and food poisoning from milk and other food products have been reported in South Africa (Prinsloo, 2001; O'Ferrall-Berndt, 2003). Milk contamination can occur during production, packaging or transportation (Dungan and Leytem, 2009; Lefoka, 2009). Legal standards have been developed worldwide to monitor compliance of microbial contaminants in milk.

Table 1.1: Number of milk producers and milk production per province (1994-1999)

	Producers		Production	
Province	**Number**	**%**	**Milk output (Million litres)**	**%**
Western Cape	1437	23	354	23
Eastern Cape	522	10	215	14
Northern Cape	100	2	15	1
KwaZulu-Natal	479	8	246	16
Free State	1505	24	277	18
North West	1144	18	185	12
Gauteng	236	4	62	4
Mpumalanga	634	10	169	11
Limpopo	63	1	15	1
Total	6220	100	1538	100

Adapted from: Dairy Development Initiative (1999).

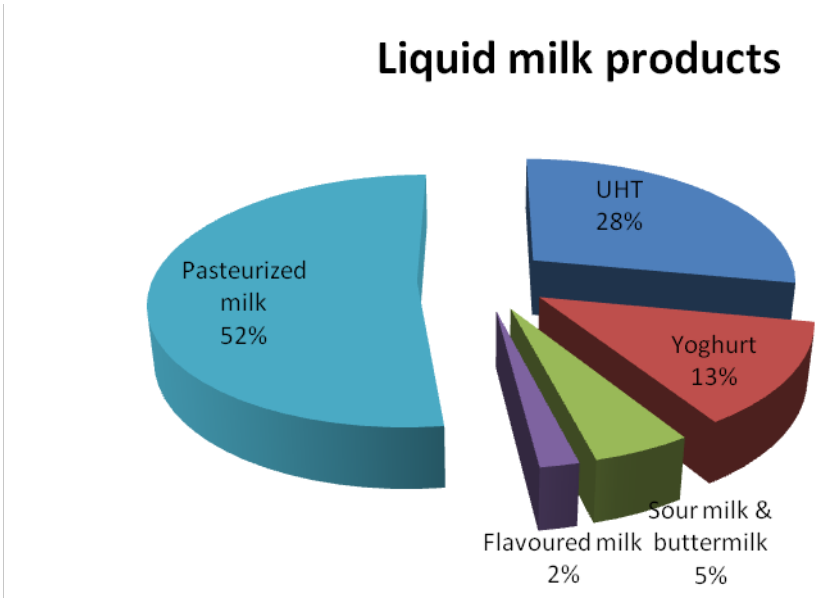

Figure 1.1: Milk products produced in South Africa (DAFF, 2011)

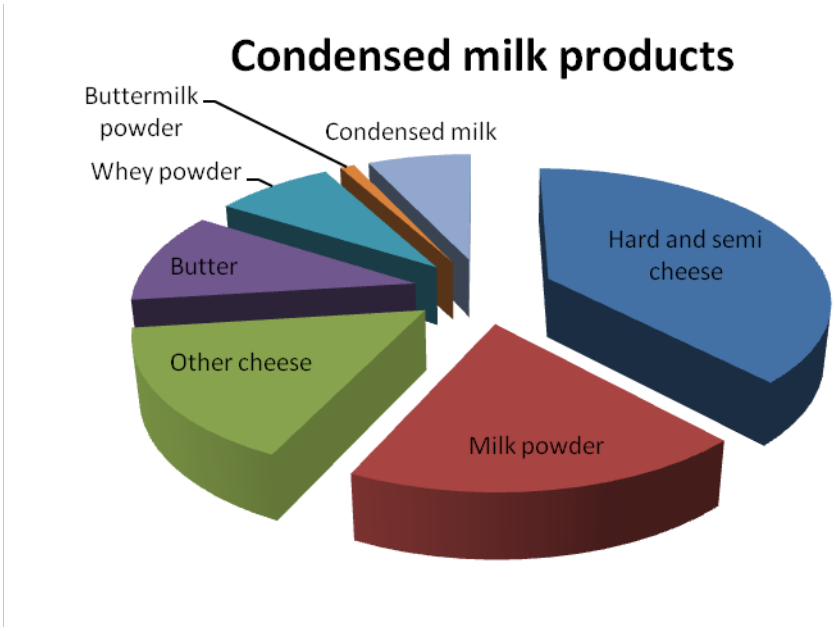

Figure 1.2: Condensed milk products produced in South Africa (DAFF, 2011)

In South Africa, the “Regulations Relating to Milk and Milk Products”, R 1555 in the Foodstuffs, Cosmetics and Disinfectants Act 54 (1972), define the standards for raw milk (Table 1.2). These standards are used to prevent contamination of milk and dairy products by harmful pathogens thus ensuring the quality of the milk. According to South African National Standards 10049 (2012), good hygiene management is needed in all aspects of the dairy plant; from the food handlers hygiene practices to the premises itself. The level of contaminants inside the milking parlour and the health of the cow may negatively affect the quality of milk and dairy products (Harding, 1995). Cross-contamination of the final product could occur if applicable measures are not adhered to. Contamination has the potential to lead to the growth of pathogenic microorganisms, resulting in disease outbreaks when the final product is consumed.

Both milk and dairy products, including cheese, are considered to be of ‘high care’ status since they do not require any further cooking prior to consumption (Foodstuffs, Cosmetics and Disinfectants Act, 1972). Cheese is a popular dairy product due to its nutritional value, convenience, taste and variety of uses (Farky, 2004). Cheese varieties are manufactured by dairy industries worldwide and are classified as soft, semi-soft, semi-hard or hard cheeses (Gregerson, 2009). In South Africa, about 82 000 metric tons of cheese are produced from 800 million litres of milk annually. In total, cheddar cheese makes up 31%, gouda makes up 20% and 49% is a combination of mozzarella, feta and cream cheese (Cheese SA, 2012). Soft cheeses such as cottage cheese or cream cheese are manufactured from lactic acid curds and characterised by high moisture content, smooth texture with a mild acidic flavour and limited shelf life (Scott, 1998; Drake *et al*., 2009). In South Africa, it is estimated that cheese consumption has increased from 1 to 9 kg per capita per year, since 1995 (International Dairy Federation, 2007). The intrinsic and extrinsic methods will be used to analyze the interaction of parameters such as pH, temperature etc. within the cottage cheese manufacturing plant.

Table 1.2: Raw milk standards in the Republic of South Africa

Analysis	**Raw cow's milk**	**Raw goat's milk**
Antibiotics	MRL levels	MRL levels
Pathogens	0	0
Total plate count	<200 000/ml	<200 000/ml
Coliform count	<20 cfu/ml	<100 cfu/ml
E. coli	0	0
Somatic cell count	<500 000/ml	<750 000/ml

Adapted from: Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972

1.2 ROLE OF MILK COMPOSITION IN CHEESEMAKING

In Africa, traditional cheese manufacturing is small scale, dictated by tradition and dependent on milk availability (O'Connor, 1993). During cheese manufacturing, pasteurized or raw milk is used. Cheeses produced from raw milk tend to have shorter ripening periods specifically because of high levels of enzymes indigenous to the milk e.g. proteases and lipases. If pasteurized milk is used, ripening periods tend to be longer. Proteases and lipases are the most common enzymes in milk, however, their presence within cold stored milk is unfavourable because proteases degrade proteins and lipases degrade fats (Mara and Kelly, 1998; Kosikowski and Mistry, 1999). During cheesemaking, nearly 10% of the fat is lost due to whey drainage. The distribution of fatty acids within milk is based on the physical nature of fatty acids as short or long chained molecules (Fox and McSweeny, 1998).

Cheeses made from full-fat milk have lower moisture content than those made from skim milk because skim milk contains more proteins and non-fat solids (Scott, 1998). Cheese flavour depends primarily on the protein profiles as well as the initial fatty acids within the milk (Scott, 1998; Fox and McSweeny, 1998; Fox *et al*., 2004). The shelf-life of the cheese depends on the sodium chloride and sugar content which inhibits microbial growth and survival on cheese in several ways (Fox *et al*., 2004). Proteins such as casein, α- lactalbumin and β- lacto globulin play an essential role in cheesemaking as it helps with the development of texture, ripening and fermentation processes (Paz *et al*., 1998; Scott, 1998). Manufacturing of other cheeses such as cottage cheese and cream cheese involves acid precipitation of casein with lactic acid or lactic acid producing microorganisms. Lactic acid bacteria (LAB) are mainly used in cheeses during the fermentation process. Lactic acid bacteria includes the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*. Lactic acid bacteria also help to suppress the growth of spoilage microbes and pathogens by producing antimicrobial substances such as bacteriocins, diacetyl and hydrogen

peroxide (Scott, 1998; Jay, 2000; Noordiana *et al*., 2013). Different kinds of cheeses include: Mozzarella, Dutch Gouda, Swiss, Romano, Blue, Goat, Camembert, Cheddar, Feta, Cottage and Ricotta (Table 1.3; Parker, 2003; Gregerson, 2009) and are classified according to soft, semi-soft, semi-hard and hard cheeses. Soft cheeses such as cottage cheese and cream cheese are typically consumed fresh.

1.3 COTTAGE CHEESE

Cottage cheese is a soft, unripened, mildly acidic cheese with high water content, limiting its shelf life (Scott, 1998; Kosikowski and Mistry, 1999; Patrick *et al*., 2004). Manufacturers of cottage cheese have developed methods that help maintain a consistent flavour, texture and appearance by the addition of *Bifidobacterium*, nisin and potassium sorbate during manufacturing (Korhonen *et al*., 1998). Ingredients of cottage cheese commonly include cultured fat free milk, lemon juice (citric acid), salt, lactic acid, natural flavouring, potassium sorbate and enzymes (Yilma *et al*., 2005; Eyassu, 2013). The milk compositions of different cottage cheeses are illustrated in Table 1.4. Several names are used in different countries for cottage cheese: in Ethiopia (Metata Ayib), in Egypt (Karish or Kariesh), in the United States (Bakers), in the United Kingdom (Cream cheese), in Germany (Quarg), in Denmark (Ymer), and in France (Fromage). Generally, cheese manufacturing involves a number of steps which are common to most cheese types. The main steps in the production of semi-soft and soft cheeses, such as cottage cheese, are outlined in Fig. 1.3.

Table 1.3: Cheese categories

Soft cheese	Semi-soft cheese	Semi-hard cheese	Hard cheese
Brie	Feta	Cheddar	Asiago
Camembert	Dutch	Colby	Blue
Cottage	Gouda	Swiss	Mozarella
Cream	Havarti	Ricotta	Parmesan

Adapted from: Gregerson, 2009

Table 1.4: Different milk compositions within cottage cheeses

Cheese type	Water (%)	Energy (kcal)	Protein (g)	Fat (g)	Ca (g)	Cholest (mg)	Poly (g)	Mono (g)	Na (mg)
Creamed cottage cheese large curd	79	235	28	10	135	34	0.3	2.9	911
Creamed cottage cheese small curd	79	215	26	9	126	31	0.3	2.7	850
Creamed cottage cheese with fruit	72	280	22	8	108	25	0.2	2.2	915
Cottage cheese low fat 2%	79	205	31	4	155	19	0.1	1.2	918
Uncreamed cottage cheese	80	125	25	1	46	10	0	0.2	19

Cal: calcium; Poly, polysaccharide; Mono, Monosaccharide; Cholest, cholesterol; Na, sodium. Adapted from: Parker, 2003

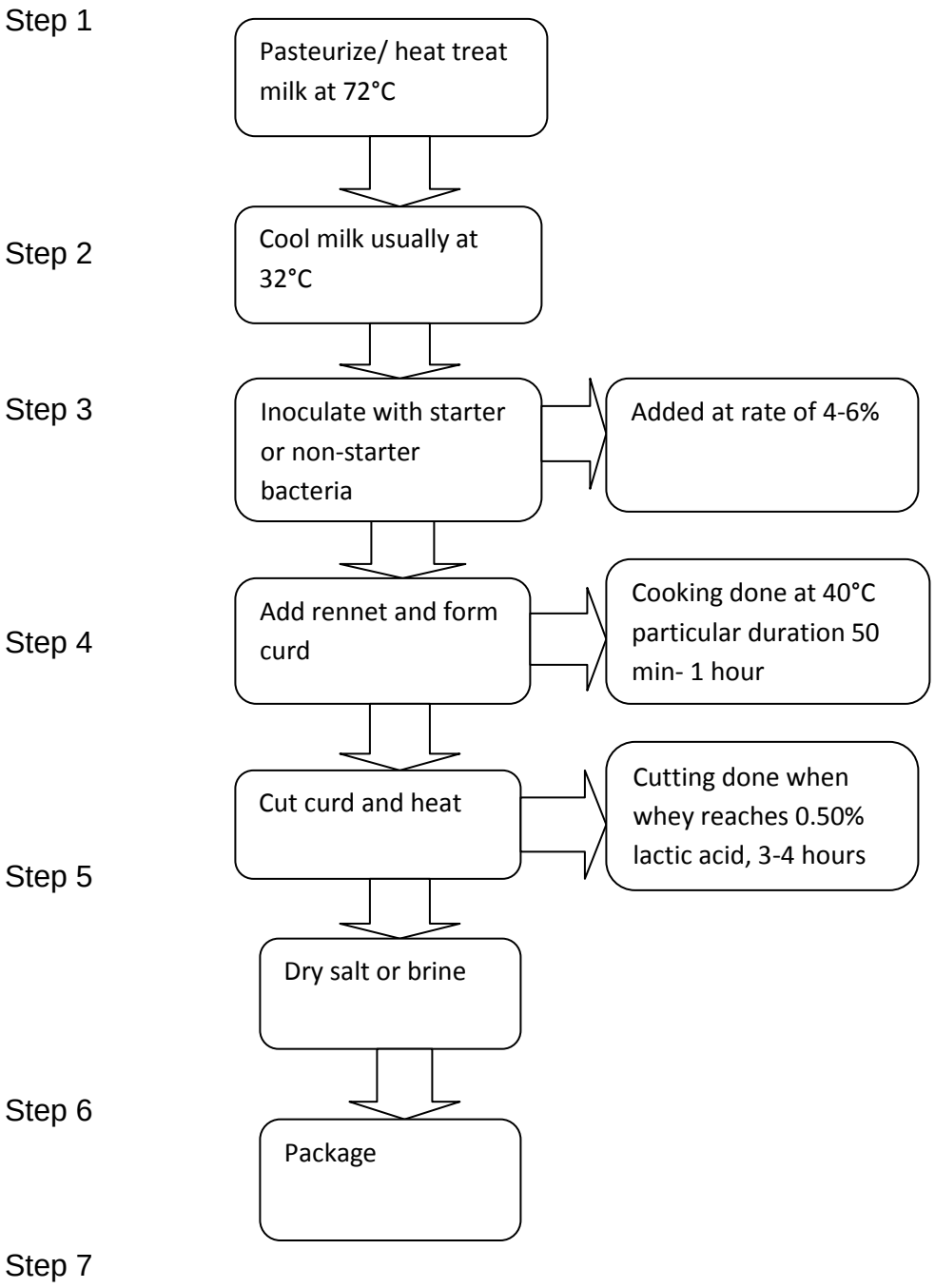

Figure 1.3: General flow diagram for cottage cheese manufacturing (Fox *et al*., 2004)

Milk used is pre-treated or pasteurized. The pre-treated or pasteurized milk is cooled at 32ºC as this temperature allows the starter bacteria to grow. Inoculation of non-starter and starter bacteria initiates bacterial growth and fermentation starts. This lowers the pH and allows the cheese flavour to develop. Addition of rennet is done to help with the formation of curd from milk proteins. Milk is curdled and drained which assists in removing whey from the cheese. Pressing is used to harden the cheese and during cottage cheese production it will extract the remainder of the whey (USDA, 2001). Lamprell (2006) suggests rinsing of the curds as this reduces the acidity of the cheese to obtain a flavour that is less sour. Cutting of the curd must be done in approximately 3-4 hours when the whey reaches 0.50% lactic acid.

The production of cottage cheese utilises the following lactic acid bacteria: *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis biovar diacetylactis*, *Lactococcus lactis* ssp. *lactis* and *Leuconostoc mesenteroides* ssp. *cremoris* (International Dairy Federation, 1991). In some cases, dry salt or a salt water solution called brine is added to the cut pieces of curds. Packaging of the cheese is done as the last stage by either waxing or packaging (Vecchionacce *et al*., 1978). Possible contamination of the cheese could occur from different areas such as the contaminated hands of food handlers, the manufacturing equipment used, the possible airborne microorganisms (bioaerosols), water used in the dairy plant, volatile organic compounds from cleaning detergents and from the preparation surfaces (Fleming-Jones and Smith, 2003; Doyle, 2007). In order to detect spoiled cottage cheese, the following serves as an indication: off-colour, slime formation and off-odours (Doyle, 2007).

1.4 MICROBIAL PATHOGENS ASSOCIATED WITH COTTAGE CHEESE SPOILAGE

Cheeses which are produced from raw milk tend to have shorter ripening periods. For the production of soft cheeses such as cottage cheese, the use of pasteurized milk is

essential (Kosikowski and Mistry, 1999). Pasteurized milk contains fewer spoilage microorganisms because most of the microbes are destroyed at 80°C for 30 minutes on small-scale production and 90°C for 5 seconds on a larger scale (Galloway, 1995; Nelson and Barbano, 2005; Gammariello *et al*., 2008). Factors that affect cheese spoilage are water activity, pH, salt to moisture ratio, temperature, characteristics of the lactic acid bacteria used as starter culture, types and viability of contaminating microorganisms, and characteristics and quantities of residual enzymes (Melilli *et al*., 2004).

High brining temperature and low initial salt determines the growth of coliform, leading to gas formation in the cheese (Melilli *et al*., 2004). Microorganisms that are associated with the spoilage of dairy products including cottage cheese are: *Staphylococcus* spp*., Salmonella* spp*., Escherichia coli, Listeria monocytogenes, Pseudomonas* spp*., Streptococcus* spp*, Leuconostoc* spp*, Lactobacillus* spp*.,* yeasts and moulds (Canganella *et al*., 1998; Gulmez and Guven, 2003; Tekinşen and Őzdemir, 2006). Some microorganisms such as lactic acid bacteria are beneficial during the production of cottage cheese and some have negative impacts on the quality, shelf life and butyric acid fermentation of the cheese (Stadhouters, 1990; Soliman and Aly, 2011).

1.4.1 Gram-negative microorganisms

Gram-negative bacteria are characterised as small, rod-shaped, facultative anaerobes such as *Escherichia coli*, *Salmonella*, *Enterococci* and *Pseudomonas.* Gram-negative bacteria are able to ferment sugars in anaerobic conditions and use a range of organic molecules in aerobic conditions (Seifert *et al*., 1993; Yamamoto *et al*., 1999). Coliforms are referred to as members of the *Enterobacteriaceae* family such as *Escherichia*, *Klebsiella* and *Enterobacter* which ferment lactose (Quinn *et al*., 2002; Asamenew *et al*., 2013). During the ripening of soft, mould-ripened cheeses the pH increases, potentially allowing the growth of coliform bacteria (Frank, 2001). Contamination of cheeses by opportunistic pathogens such as *Escherichia coli* (*E.coli*) indicates faecal contamination

(Jay, 2000; Blanco *et al*., 2003; Mora *et al*., 2007). *Escherichia coli* are commonly found in the normal microflora of the intestinal tract of humans and other mammals. Most strains of *E. coli* are harmless but some causes diarrheal diseases as they are pathogenic (Meng *et al*., 2001). Enteropathogenic *E. coli* is considered a good indicator of faecal contamination and its presence within milk and dairy products constitutes a public health hazard (Griffin and Tauxe, 1991).

Salmonella spp. contaminate a wide range of food such as poultry, raw meats, milk and dairy products leading to food poisoning. *Salmonella* causes the disease called salmonellosis. The primary source of infection is through the ingestion of food contaminated with salmonella bacteria. The most prevalent organism causing acute infectious disesase from contaminated foods is *Salmonella typhi*. *Salmonella typhi* is known to ferment glucose with the production of gas and acid (Cliver, 1990; Forsythe, 2000). *Enterococci* presence in dairy products has long been associated with inadequate sanitary conditions during milk processing and production (Giraffa *et al*., 1997). *Enterococci* microorganisms are resistant to antibiotics and are sometimes associated with hospital environments (Deibel and Siliker, 1963). *Pseudomonas* spp. such as *Pseudomonas auruginosa*, *Pseudomonas putida* and *Pseudomonas fluorescens,* amongst others, are commonly isolated from soil. Some species of *Pseudomonas* are psychrophilic as they grow at temperatures below 5°C and others are adapted for growth at ambient warmer temperatures. Due to the high moisture content of 50%-80% and pH values of 5.0-6.5, spoilage of soft cheeses can occur by *Pseudomonas, Alcaligenes* and *Flavobacterium* (Lycken and Borch, 2006; Doyle, 2007).

1.4.2 Gram-positive microorganisms

Gram-positive microorganisms are classified as strict or facultative anaerobes. The most common Gram-positive bacteria include *Bacillus* spp., *Lactobacillus* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Clostridium* spp. The majority of pathogens in humans are Gram-positive bacteria. *Bacillus* and *Clostridium* spp. are

characterised as bacilli that make protected spores causing anthrax, gastroenteritis, botulism, tetanus and gas gangrene (Tauxe, 2002; Doyle, 2007; Woodford and Livermore, 2009). Staphylococcal food poisoning is caused by the ingestion of enterotoxins secreted by this pathogen. Staphylococcal species originate from normal human microflora, nose, oropharynx and mouth. *Staphylococcus* food poisoning is most frequently from milk and dairy products, meat and meat products as well as bakery products (Cliver, 1990). *Streptococcus* spp. produce haemolytic enzymes that cause the souring of milk and dental decay and are often found in the throats and on the skins of people even though there may be no visible symptoms (Schleifer *et al*., 1995). *Lactobacillus* spp. are characterised by their ability to produce lactic acid as a by-product of glucose metabolism. *Lactobacillus* is widely distributed in animal feeds, manure, milk and milk products. Various species of *Lactobacillus* are commercially used during production of cheeses, sour milks and yoghurt. Finally, *Clostridium sporogenes* spoils processed cheese by producing off-flavours and gas holes (Lycken and Borch, 2006).

1.4.3 Yeasts and moulds

Yeasts are single-celled eukaryotic microorganisms classified in the kingdom fungi. Yeasts adapt to specialised and liquid environments without producing toxic secondary metabolites (Pitt and Hocking, 1999; Smits and Brul, 2005; Kurtzman, 2006). Yeasts typically grow in moist environments and are also useful in the fermentation of bread, beer and many dairy products. Heterogeneous yeast groups such as *Candida* and related genera are also involved in the spoilage of some dairy products, vegetables and fruits leading to human infections when consumed (Casey and Dobson, 2003; Fitzgerald *et al*., 2004). Yeast species frequently isolated from cheese are *Candida lipolytica* and *Debaryomyces hansenii* (Nakase *et al*., 1977; Viljoen, 2001). *Debaryomyces hansenii* regularly occurs in salt brines used for cured meats, olives and cheese due to its ability to grow in high salt concentrations of 24% (Castro *et al*., 2003; Martorell *et al*., 2005; Lycken and Borsch, 2006; Martorell *et al*., 2007). Several species of *Candida, Cryptococcus*, *Debaryomyces*, *Geotrichum*, *Rhodotorula* and

Saccharomyces have been found to be common spoilage yeasts in dairy products (Westall and Filtenborg, 1998; Lopandic *et al*., 2006). Dairy products and typical types of spoilage microorganisms are shown in Table 1.5.

1.5 OTHER CONTAMINANTS ASSOCIATED WITH COTTAGE CHEESE SPOILAGE

In the past, contamination of dairy products was known to occur by contact with soiled surfaces, but it is now proven that bioaerosol contaminants also play a major role (Heldman, 1974; Mokoena, 2013). Bioaerosols originate from microorganisms (bacteria, moulds, viruses) and pollen, particularly as volatile organic compounds (VOCs) that can be odourless (Schiff *et al*., 2000). Bioaerosols co-exist in the air as tiny droplets of water and are distributed throughout a room according to airflow patterns (Goyer *et al*., 2001; Shale and Lues, 2007). Bioaerosol contamination of food and milk products, equipment surfaces and containers utilised during processing can aggrevate the spread of infections (Radon *et al*., 2002). Pathogens attach to dust particles and condensation facilitating the spread of bioaerosols within the processing facility. Exposure to and inhalation of bioaerosol pollution is associated with a wide range of health effects including acute toxic effects, allergies, cancer and infectious diseases (Flannigan *et al*., 1991; Crook and Sherwood-Higham, 1997; Cullinan *et al*., 2001). Factors like meteorological changes in temperature, airflow velocity and relative humidity affect the dispersion, viability and concentration of airborne microbes (Jones and Harrison, 2003).

The generation of heating, ventilation and air conditioning systems (HVAC) in processing facilities further contributes towards increasing numbers of airborne microorganisms as they offer ideal growth conditions (Heldman, 1974; Rahkio and Korkeala, 1997; Downes and Ito, 2001).

Table 1.5 Dairy products and typical types of spoilage microorganisms or microbial activity

Food	Spoilage microorganism or microbial activity
Raw milk	A wide variety of different microbes
Pasteurized milk	Psychrotrophs, sporeformers, microbial enzymatic degradation
Concentrated milk	Spore-forming bacteria, osmophilic fungi
Dried milk	Microbial enzymatic degradation
Butter	Psychrotrophs, enzymatic degradation
Cultured buttermilk, sour cream	Psychrotrophs, coliforms, yeasts, LAB
Cottage cheese	Psychrotrophs, coliforms, yeasts, moulds, etc.
Yoghurt, yoghurt-based drinks	Yeasts
Other fermented dairy foods	Fungi, coliforms
Cream cheese, processed cheese	Fungi, spore-forming bacteria
Soft, fresh cheeses	Psychrotrophs, coliforms, fungi, LAB, etc.
Ripened cheese	Fungi, LAB, spore-forming bacteria, etc.

LAB: Lactic acid bacteria, Adapted from: Lempert, 2004.

In dairy operations, there is the potential for offsite contamination and subsequent transport of bioaerosols (Wilson *et al*., 2002; Green *et al*., 2006; Heederik *et al*., 2007). Airborne microorganisms in the food and dairy industry have long been recognized as potential contaminants resulting in low quality products with a reduced shelf life. Sources of bioaerosols within the dairy processing facility include worker activity, sneezing, talking, coughing, HVAC systems, sink and floor drains and operation of equipment (Kang and Frank, 1990; Ren and Frank, 1992; Cosenza, 2004). Inhalation of bioaerosols can cause infections like pneumonitis and inhalation of odourants from VOCs has elicited health problems such as increased tension, fatigue, mood changes and depression (Cole *et al*., 1999; Schiffman *et al*., 2000; Goyer *et al*., 2001).

Certain VOCs perceived as odourous are not necessarily associated with presence of faecal indicator microorganisms or pathogens (Schiffman and Williams, 2005). However, even when VOCs are only detectable at exceedingly low concentrations they can accumulate rapidly at intensive animal stocking densities, causing respiratory hazards for animals and workers (Millner and McConnell, 2000). VOCs can be products of chlorination of drinking water and combustion, with some VOCs acting as food additives from commercial packaging components (Dauneau *et al*., 1997). VOCs in cheese and bacterial suspensions were determined using gas chromatography- mass spectrometry (GCMS) (Canac-Artega *et al*., 1999).

1.6 MICROBIOLOGICAL ANALYSIS

There are several techniques used for the analysis of bioaerosols and organic acid profiles such as gas chromatography- mass spectrometry (GC-MS), Limulus (LAL), bioassays, culturing, Bioaerosols Mass Spectrometry (BAMS), Polymerase Chain Reaction (PCR), High Performance Liquid Chromatography (HPLC) and Matrix Assisted Laser Desorption/Ionisation Time Of Flight Mass Spectrometry (MALDI TOF MS) (Conway *et al*., 2001; Smole *et al*., 2002; Friedrichs *et al*., 2007; Mellmann *et al*., 2008). MALDI TOF MS is used for the identification of bacteria or fungi. It is a focused laser

beam, either in the Ultra Violet or Infra Red range that is pulsed onto crystals on a target steel plate which absorbs the laser energy, becomes partially vaporised and carries intact DNA into the vapour phase (Seng *et al*., 2009). The matrix crystals do this by transferring part of their charge to the DNA of the organism, allowing for ionization, while protecting the DNA from degradation (Simpson *et al*., 1999). MALDI TOF MS can identify both Gram-negative and Gram-positive bacteria down to species level. In addition, MALDI TOF MS is able to measure other bacterial components and molecular masses of proteins from whole bacterial extracts (Conway *et al*., 2001; Friedrichs *et al*., 2007, Mellmann *et al*., 2008).

High Performance Liquid Chromatography (HPLC) separates compounds based on their solubility in particular solvents. HPLC methods have been used to quantitate amines in cheese, fish and chicken (Baixas-Nogueras *et al*., 2005; Balamatsia *et al*., 2006; Innocente *et al*., 2007). Combined determination of pH values affecting the chromatographic behaviour of organic acids in cheese and other food compounds is analyzed using an ion-exchange or reverse-phase HPLC (Marsili *et al*., 1981; Zhao *et al*., 2001; Fox *et al*., 2004). Reverse-phase chromatography can be used to separate all the components of cheese, i.e. a- ketoisocaproate, 3-methyl-2-oxovalerate, 3-methyl-2-oxobutyrate, 4-methylthio-2-oxobutyrate, b-phenylpyrovate, and r-hydroxyphenyl pyrovate. The production of these keto acids is related to aminotransferase activities. In addition to non-volatile acids such as citric-, orotic-, piruvic-, lactic- and hippuric acid, sugar concentrations of lactose, glucose and galactose can be determined in cheese using HPLC. For flavour analysis HPLC is not often used, as most flavours are directly amenable to gas chromatography (GC) analysis (Marilley & Casey, 2004). However, Zeppa *et al*. (2001) and Akalin *et al*. (2002) used HPLC to evaluate variations of organic acids during ripening of cheese. Although several instrumental channels can be used in parallel with HPLC, the risk of spoiling the chromatography column is relatively high with complex microbiological samples.

1.7 RATIONALE

A leading manufacturer of cottage cheese in South Africa has been experiencing problems with spoilage of its products. Literature studies on cottage cheese in South Africa are rare and most of them are not recent, which is why this study is relevant. Therefore, the aim of this case study was to determine and evaluate the effects of airborne contaminants and their distribution, extrinsic and intrinsic parameters during the production of cottage cheese, as well as to determine and assess the microbial contaminants from surface equipment which may affect its quality and jeopardise the shelf life of cottage cheese.

In order to achieve this aim, the objectives were as follows:

- To quantify and identify airborne and surface microorganisms and related VOCs during cottage cheese production.
- To assess microbial and organic acid concentrations as well as to determine the shelf life of cottage cheese.
- To measure the related environmental parameters and to evaluate their effects during cottage cheese production.
- To conduct a survey on food handlers in cottage cheese production in order to determine the practices, knowledge and behaviour during production of cottage cheese.

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BIOAEROSOLS AS A POSSIBLE SOURCE OF CONTAMINATION DURING COTTAGE CHEESE PRODUCTION

CHAPTER 2

ABSTRACT

Airborne particles are a potential source of fungal and bacterial spores. Measurements of bioaerosols, temperature, wind velocity and relative humidity were performed from four different areas within the cottage cheese section of a dairy plant. Airborne samples were collected from the: processing area, packaging area, shrink wrap area and pasteurization area. Airborne microbes were cultured and identified through the use of Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS). Average temperatures from the shrink wrap and processing areas ranged from 20.9°C to 25.2°C and wind speed averaged 0.6 $m.s^{-1}$ to 1.0 $m.s^{-1}$, with average relative humidity between 41% and 68%. Bacterial counts in the cheese processing area had a maximum value of 92 $cfu.ml^{-1}$ compared to other parts of the cottage cheese section. Commonly identified bacteria included *Micrococcus luteus*, *Acinetobacter* spp., *Pseudomonas* spp., *Kocuria* spp., *Staphylococcus* spp., *Enterococcus* spp. and yeast such as *Candida* spp. *Acinetobacter* spp. and *Kocuria* spp. pose a possible health risk to immune compromised people. *Pseudomonas* spp., *Staphylococcus* spp. and *Candida* spp. cause food spoilage and disease in humans. It is therefore necessary to properly measure airborne contaminants to minimise their spread.

Keywords: *bioaerosols, cottage cheese, MALDI TOF MS, environmental parameters, South Africa.*

2.1 INTRODUCTION

Bioaerosols are airborne microbial contaminants that are universal in nature and can be harmful to humans and animals and also negatively affect the quality and shelf life of food and beverage products (Griffiths *et al*., 1997; Wirtanen *et al*., 2003; Shale and Lues, 2007). Microbial particles are generated at a source and distributed throughout a room according to the airflow patterns (Shale and Lues, 2007). Airborne microorganisms in the food industry, including the dairy industry, have long been recognized as potential food contaminants (Radmore, 1986; Kang and Frank, 1989). Bioaerosol contamination of food and milk products can cause or promote the spread of infections (Radon *et al*., 2002). Contamination of milk products, particularly cheeses, are typically due to the presence of moulds and yeasts in cheese manufacturing environments such as walls, air, shelves of ripening rooms, water, equipment and milk (Chapman and Sharpe, 1990; Jay, 1992; Shale and Lues, 2007; Lecours *et al*., 2012).

The organoleptic characteristics of the cheeses can be influenced by the wild types of moulds present in the processing environment (Jordal *et al*., 1993; Wouters *et al*., 2002). Common spoilage microorganisms found in milk and dairy products include *Pseudomonas* spp., coliforms, *Bacillus* spp., *Clostridium* spp., lactic-acid bacteria and enterococci (De Buysier *et al*., 2001; Albert *et al*., 2007; Lecours *et al*., 2012). The number of spoilage microorganisms identified depends on several factors including the quality of starter cultures, quality of the rinsing water and the general cleanliness of the plant (Bramley and McKinnon, 1990). Current methods for analyzing bioaerosols include Limulus (LAL) bioassay, culturing, Bioaerosol Mass Spectrometry (BAMS) and Polymerase Chain Reaction (PCR). Matrix Assisted Laser Desorption/ionization Time Of Flight Mass Spectrometry (MALDI TOF MS) is able to measure molecular masses of proteins obtained from the whole bacterial extracts and allows for the rapid identification of both Gram-positive and Gram-negative bacteria to species level (Conway *et al*., 2001; Smole *et al*., 2002; Friedrichs *et al*., 2007; Mellmann *et al*., 2008). Therefore, this study aimed to identifying airborne microorganisms (bioaerosols) and their sources during cottage cheese production in a dairy plant in Bloemfontein, Central South Africa.

2.2 MATERIALS AND METHODS

2.2.1 Collection of samples

Samples were collected from the cottage cheese section of a dairy plant in Bloemfontein, Central South Africa. Measurements of bioaerosols, temperature, airflow, wind velocity and relative humidity were performed from four different areas within the cottage cheese section of a dairy processing facility (Fig 2.1). Airborne samples were collected from the processing area, packaging area, shrink wrap area and pasteurization area. Air samples were collected aseptically in duplicate using a Surface Air Sampler (SAS) Super 90 machine (PBI International, Milan, Italy). The Plate Count Agar (PCA) plates were incubated at 37°C for 24 hours and PDA (Potato Dextrose Agar) plates were incubated at 25°C for 72 hours. Once the organisms had grown, plates were put under a light counting machine (Vacutec, Randburg, South Africa) and colonies counted. Some colonies were transferred to Nutrient Agar (NA) plates as single colonies. The NA plates were incubated at 37°C for 24 hours for further bacterial growth. The fungi isolated from PDA plates were only counted and MALDI extraction method was used for the identification of yeast and mould species.

2.2.2 Evaluation of samples by environmental parameters

A Tempstress meter (Ques Temp °32, Quest Technologies Inc., Oconomowac, WI) was used to measure the temperature as well as the relative humidity. Distilled water was poured into a Wet Bulb Globe Temperature (WBGT) piece, with the tripod set at a height of 1.5 m for standing workers. The machine was left to acclimatize to the environment for approximately 30 minutes in the area to be tested. Three samples were taken at intervals of 15 minutes. A vane anemometer (Airflow Instrumentation LCA 6000 VT, High Wycombe, Buckinghamshire, UK) was also used to measure the average and maximum airflow velocity rate (wind speed) in all four sampled areas.

2.2.3 Analysis using MALDI TOF MS fingerprinting

Airborne microbes were cultured and subsequently identified using MALDI TOF MS (Bruker Daltonics, Germany). Pure colonies detected from the NA plates were first used for MALDI TOF MS identification (Van Wuijckhuijse *et al*., 2005). If the results were not conclusive or peaks could not be detected, then preparation for the Ethanol/formic acid extraction method was performed. Isolated microorganisms (5-10 mg) were carefully removed from the plate and transferred into Eppendorf tubes, filled with 300 µl of double distilled water (Merck, South Africa) and thoroughly mixed. Absolute ethanol (900 µl) was added to each tube and centrifuged at 13200 rpm at room temperature for 2 minutes. The residual ethanol was completely removed by careful pipetting and pellets left to dry at room temperature. Seventy percent formic acid (50 µl) was added to the dry pellets and mixed by vortexing, followed by the addition of pure acetonitrile (50 µl) to the solution. After careful mixing, the solution was centrifuged at 13200 rpm for 2 minutes. Approximately 1 µl of the supernatant was placed onto a 96 polished steel target plate (Bruker Daltonics, Germany) and allowed to dry at room temperature. Afterwards, each sample was overlaid with 1 µl of the HCCA matrix solution (α-Cyano-4-hydroxycinnamic acid, portioned, Sigma, USA) and air dried at room temperature.

The analyses of all strains were done on a Microflex LT mass spectrometer (Bruker Daltonics) using Flex Control software (Version 3.0). The spectrum was recorded with the use of a laser frequency of 20 Hz, an ion source of 1V (20 kV); ion source of 2V (18.6 kV); lens voltage of 7.5 kV and a mass range of 2000 to 20 000. All the analyzed spectra were internally calibrated using *Escherichia coli* proteins as standard. The BioTyper software (Version 3.0, Bruker Daltonics, Germany) was used for the detection of raw spectra with matching standard pattern and settings. Results were reported in a ranking table identified by a set of colour codes (green, yellow and red). Identity (ID) scores ranged from 0 to 3. Outcomes were expressed as proposed by MALDI TOF biotyper (MT). The scores of <1.7 were considered unreliable; whereas scores of 1.7<ID<1.9 were considered ID to genus level only and scores of >1.9 were used for reliable species ID.

2.3 RESULTS AND DISCUSSIONS

2.3.1 Gram-negative isolates from areas sampled in the cottage cheese section

The bacterial and fungal counts within the four sampled areas in this study, namely: processing area, pasteurization area, shrink wrap area and packaging area are shown in Fig 2.1. *Acinetobacter* species (*A. baumanii, A. johnsonii, A. junii*, *A*. *lwoffii*) were the most predominant Gram-negative bacteria isolated from all sampled areas (Table 2.1). *Acinetobacter* species are commonly found in soil, dust and on human skin. Species of *Acinetobacter* have also been isolated from bioaerosols at a concentrated dairy operation sites (upwind and downwind operations) and have been associated with nosocomial infections (Manfredi *et al*., 2001; Smolyakov *et al*., 2003; Dungan *et al*., 2010). Other Gram-negative bacteria isolated were *Agrobacterium tumefaciens*, *Pseudomonas jessenii*, *Pseudomonas veronii, Pseudomonas aeruginosa* and *Salmonella enterica* (Table 2.1). *Pseudomonas aeruginosa* has been implicated in foodborne outbreaks and has previously been isolated from cottage cheese (Singh and Prakash, 2008). *Pseudomonas jessenii* strain CIP 105274 which was identified from this study through aerosols, has been isolated from natural mineral waters in France (Verhille *et al*., 1999) while *Pseudomonas veronii* is commonly used in the bioremediation of contaminated soils and is capable of degrading a variety of aromatic organic compounds (Elomari *et al*., 1996).

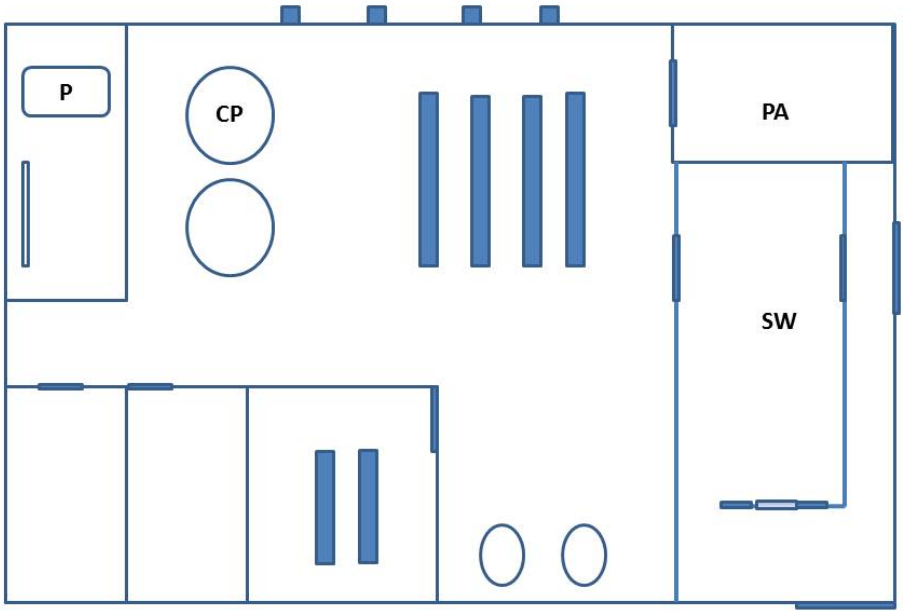

Figure 2.1: Cottage cheese section showing different areas sampled.

P- Pasteurization area, CP- Cheese processing area, PA- Packaging area, SW- Shrink wrapping area.

Table 2.1: Gram-negative isolates from sampled areas

ISOLATED SPECIES	Isolated Area	SOURCE	IMPLICATIONS	REFERENCES
Acinetobacter sp LMG 1300 HAM *A. baumanii* DSM 3007T HAM *A. johnsonii* DSM 348 DSM *A. junii* DSM 6964T HAM *A. lwoffii* DSM 2403T DSM	Processing area Packaging area Shrink wrapping area Pasteurization area	Soil Water Human skin Dust Oropharynx	Infection in debilitated patients Nosocomial pneumonia Rare cases of bloodstream infection	Seifert *et al*., 1993,
Agrobacterium tumefaciens DSM 30147T HAM	Pasteurization area	Soil	Causal agent of crown gall disease, tumours	Young *et al*., 2001
Aromatoleum aromaticum EbN1 MPB	Packaging area	Freshwater and soil habitats	Capable of microbial biodegradation	McLeod and Eltis, 2008
Burkholderia tropica DSM 15359 HAM	Processing area	Soil, maize, sugarcane	Plant pathogens	Reis *et al*., 2004
Breundimonas vesicularis DSM 7226T HAM	Processing area	Fresh aquatic environments	Rarely isolated from clinical specimens	Panasiti *et al*., 2008
Chryseobacterium sp 107 PLM	Processing area	Food, plant roots, water and clinical environments	Associated with meningitis in premature neonates	Kämpfer *et al*., 2003; Kim *et al*., 2005a
Empedobacter brevis LMG 4011T HAM	Processing area	Dust, soil, water, hospital environments, raw meat products	Rare nosocomial infections	Bellais *et al*., 2002
Moraxella osloensis 76 P1M	Processing area and shrink wrapping area	Infrequently isolated from clinical specimens	Fever, petechial rash	Shah *et al*., 2000
Pseudomonas aeruginosa ATCC 27853 CHB *Pseudomonas caricapapayae* LMG 2152T HAM *Pseudomonas koreensis* 2-2 TUB *Pseudomonas jessenii* CIP 105274T HAM, *Pseudomonas veronii* B560 UFL	Processing area Shrink wrapping area Pasteurization area	Soil Natural mineral water Human skin flora Farming soil Natural springs	Causes spoilage of dairy products	Franzetti and Scarpellini, 2007
Rothia nasimurium 10036873-108 USH	Shrink wrapping area	Isolated from the nose of the mouse	None known	Collins *et al*., 2000
Salmonella enterica 11 LAL	Processing area	Human faeces, improperly prepared meats, surfaces of raw eggs, goat's cheese	Causes diarrhoea and vomiting	Van Cauteren *et al*., 2009 Zhensheng *et al*., 2009
Streptomyces phaeochromogenes B265 UFL	Shrink wrapping area	Antibiotics	Pancreatic cancer	Bum-Joon *et al*., 2004
Sphingomonas trueperi DSM 722ST DSM	Processing area	Soil	None known	Kämpfer *et al.,* 1997
Yersinia kristensenii DSM18543T RKB	Processing area	Isolated from faeces or urine of rodents	Infections may occur through consumption of contaminated milk and meat products	Hamama *et al*., 1992

Salmonella enterica was isolated from bioaerosols within the cottage cheese section in this study (Table 2.1) and has been implicated in many food poisoning outbreaks due to contaminated poultry and products that contain eggs (van Cauteren, 2009). Food poisoning due to *Salmonella* spp. in the USA alone accounts for 1.4 million infections, 15 000 hospitalization cases and 400 deaths yearly (Olson *et al*., 2007; Zhensheng *et al*., 2009).

2.3.2 Gram-positive isolates from areas sampled in the cottage cheese section

Gram-positive bacteria isolated were *Staphylococcus aureus*; *S. warneri*, *S. cohnii*, *S. hominis*, *S. scuiri, S. xylosus*, *Viridibacillus arenosi*, *Micrococcus luteus*, *Arthrobacter gandavensis*, *Enterococcus faecium*, *Enterococcus bovis*, *Enterococcus faecalis Arthrobacter creatinolyticus*, *Gordonia rubripertincta*, *Kocuria palustris*, *Viridibacillus arenosi, Bacillus agaradhearens*, *Brachybacterium faecium*, *Kocuria rhizophila*, *Lactobacillus pantheris* and *Clostridium baratii* (Table 2.2). Botulism is a fatal disease that can be caused by toxin F produced by *Clostridium baratii* (Harvey *et al*., 2002). Strains of *Clostridium* are widely distributed in the environment, forming an essential part of humans' and many animals' normal colonic microflora (Allen *et al*., 2003).

Staphylococcus aureus isolated from the production area from the present study is frequently found in the human respiratory tract and in the normal flora of the skin. This opportunistic pathogen has been implicated in food poisoning outbreaks from dairy products such as cheese, ice-cream, dried and raw milk (Torgar and Teger, 2006). *Staphylococcus aureus* contamination has also been found in cottage cheese in a study done by Singh and Prakash (2008). Other Gram-positive bacteria that contaminate dairy products include *Viridibacillus arenosi*, *Micrococcus luteus* and *Arthrobacter gandavensis* (Greenblatt *et al*., 2004). These bacteria occur mainly in soil, river sediment, dust and human blood cultures. *Enterococcus* presence in dairy products has long been associated with inadequate sanitary conditions during milk processing and production (Giraffa *et al*., 1997).

Table 2.2: Gram-positive isolates from sampled areas

ISOLATED SPECIES	ISOLATED AREA	SOURCE	IMPLICATIONS	REFERENCES
Arthrobacter gandavensis DSM 15046T DSM	Processing area	Cow teat skin and teat canal	Human skin infection	Verdier-Metz *et al*., 2012
Arthrobacter creatinolyticus DSM 15881T DSM	Pasteurization area	Isolated from human urine	None known	Hou *et al*., 1998
Bacillus licheniformis DSM 13T DSM *Bacillus pumilus* DSM 1794 DSM *Bacillus agaradhaerens* DSM 8721T DSM	Processing area Packaging area	Soil bird feathers chest and back plumage	Non- toxigenic	Salkinoja-Salonen *et al*., 1999
Brachybacterium faecium IMET 11352T HKJ	Packaging area	Isolated from poultry deep litter	None known	Gvozdyak *et al*., 2002
Clostridium baratii 1018_NCTC 10986 BOG	Packaging area	Widely distributed in the environment, colonizes the human and animal intestinal tracts	A potential human pathogen	Harvey *et al*., 2002
Enterococcus faecium VRE_PX_16086218 MLD	Processing area	Human skin	Neonatal meningitis	Laukova and Czikková, 2001
Gordonia rubripertincta DSM 43240 DSM	Processing area, shrink wrapping area and pasteurization area	Human skin	None known	Yassin *et al*., 2007
Kocuria rhizophila DSM 348 DSM *Kocuria palustries* DSM 11925T DSM *Kocuria varians* DSM 20033T DSM	Processing area Pasteurization area Packaging area shrink wrapping area	Soil fermented foods, water air	Colonize the skin, mucosa and oropharynx, brain abscess	El-Baradei, 2007
Lactobacillus coryniformis DSM 20001T DSM *Lactobacillus pantheris* DSM 15945T DSM	Processing area Packaging area	The mucosa and intestines of humans and animals fermenting foods and feed	Improves intestinal function of healthy adults and enhances immune response	Vinderolla *et al*., 2000
Micrococcus luteus IMET 11249 HKJ	Processing area, shrink wrapping area packaging area	Soil, water, animals and some dairy products	None known	Greenblatt *et al*., 2004
Staphylococcus aureus DSM 11822 DSM *S. cohnii ssp cohnii* DSM 20262 DSM *S. hominis* Mb18788_1 CHB *S. scuiri ssp scuiri* DSM 6671 DSM *Staphylococcus xylosus* F1FLR	Processing area Packaging area	human respiratory tract and on the human skin rare cause of mastitis in cattle	Diarrhoea, respiratory disease boils sinusitis	Borelli *et al*., 2006 Torgar and Terger, 2006
Viridibacillus arenosi DSM	Packaging area	forest soil	None known	Reid *et al*., 2012

Enterococcus spp. are sometimes associated with hospital settings and abdominal infections, because they are resistant to antibiotics (Centeno *et al*., 1996). *Enterococcus faecium*, *Enterococcus bovis* and *Enterococcus faecalis* are frequently isolated from contaminated fermented milk and were isolated in this study within the cottage cheese section (Birollo *et al*., 2001). *Micrococcus luteus,* a spherical and non-motile bacterium, was isolated from the processing area, shrink wrap area and packaging area (Table 2.2). More commonly isolated from soil, water, air, dust and the human skin flora, this bacterium is also known to colonize the mouth, oropharynx and upper respiratory tracts of humans. *Micrococcus luteus* is known to tolerate drying and high salt concentrations in dairy products and is an opportunistic human pathogen particularly in immune compromised people (Moriguchi *et al*., 1994; Mukamolova *et al*., 2002; Greenblatt *et al*., 2004).

2.3.3 Fungal isolates from sampled areas in the cottage cheese section

High counts of yeasts were expected within the pasteurization area due to the high humidity and moisture content caused by the activities within this area, providing favourable conditions for the growth and survival of common species of yeasts. However, the only yeast identified in this area was *Candida parapsilosis* (Table 2.3). *Candida glabrata* was isolated from the packaging area and is known as a significant human opportunistic pathogen of the mucosal tissues. *C. glabrata* is currently ranked the second or third yeast as a causative agent of superficial oral, vaginal or urinary systemic candidal infections, often resulting in nosocomial infections (Fidel *et al*., 1999). From the processing area *Candida lamblica* was isolated, a species of yeast commonly found in dairy products, fruit juices, birds and humans, where it is responsible for bloodstream infections in humans (Kurtzman and Fell, 2000). *Colletotrichum gloeosporioides* was isolated from the production area (Table 2.3) and originates from medicinal plants but does not possess any health impacts in humans (Gangadevi and Muthumary, 2008).

2.3.4 Total measurements of relative humidity, wind velocity and temperature

The average temperatures were 20.9°C and 25.2°C in the shrink wrapping and processing areas, respectively, whereas the temperatures in the packaging and pasteurization areas were 22.8°C and 22.9°C (Table 2.4). The average wind velocity for the shrink wrap area and pasteurization area ranged between 0.6 $m.s^{-1}$ and 1.0 $m.s^{-1}$ and for the processing area and packaging area ranged between 0.7 $m.s^{-1}$ and 0.9 $m.s^{-1}$. The air movement within the entire plant conformed to the minimum requirements as stipulated in the Occupational Health and Safety Act (85 of 1993) from the Environmental Health Regulations (2003) for work places and for food premises such as dairy plants. It was noted that the temperature and relative humidity within the processing area and pasteurization area had maximum values of 66% and 68%; hence the high numbers of microorganisms isolated from those areas (Table 2.4). The results obtained from the packaging area and shrink wrap area revealed minimal risks of contamination to the end product since the relative humidity ranged between 41% and 58%. The average temperatures varied from 20.9°C to 22.8°C with a wind speed of 0.6 $m.s^{-1}$ and 0.7 $m.s^{-1}$ for the packaging area and shrink wrap area.

The survival of most moulds within the processing and pasteurization areas were possible because of the favourable conditions such as high temperature, humidity and high moisture content. The control of condensation, spores, humidity as well as dust should be taken into consideration. The reduction of mould spoilage may be accomplished through ozone treatment, antimycotic coating of packaging material, and sterile filtration of air as well as ultraviolet disinfection of handling surfaces (Marriot and Gravani, 2006). Mould could be controlled by spraying chemical disinfectants in the air through routine practice. Marriot and Gravani (2006) emphasize the importance of ventilation during processing steps especially in areas where excess heat is produced. The ventilation systems should act as a barrier for dirty air and floor drains should act as a barrier for sanitation, but these surfaces and equipment may also contribute to the attachment sources of airborne microbes (Marriot and Gravani, 2006).

Table 2.3: Isolated yeasts species from sampled areas

ISOLATED SPECIES	ISOLATED AREA	SOURCE	IMPLICATIONS	REFERENCES
Candida lambica (ana) CBS 603 CBS	Processing area	Found in dairy products, fruit juice, water, birds and man	Bloodstream infections of patients with hematologic malignancies	Kurtzman and Fell, 2000
Colletotrichum gloeosporioides CBS 100471 CBS	Processing area	Leaves of a medicinal plant	None known	Gangadevi and Muthumary, 2008
Candida glabrata 31 PSB	Packaging area	Normal flora of healthy individuals	A highly opportunistic pathogen of the urogenital tract of the bloodstream, prevalent in HIV positive people and the elderly	Fidel *et al*., 1999
Candida parapsilosis ATCC 22019 THL	Pasteurization area	Frequently present in many types of cheese. Found in Italian raw milk	Sepsis of wound and tissue infections in immunocompromised patients	Cocolin *et al*., 2002; Coppola *et al*., 2001

Table 2.4: Total temperature, wind velocity and relative humidity measured

AREA	Temperature (°C)			Wind Velocity (m/s)			Relative Humidity (%)		
	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg
Packaging	23.5	22.1	22.8	1.1	0.4	0.7	61	56	58
Processing	26.5	24	25.2	1.3	0.6	0.9	75	62	68
Pasteurization	25.9	20	22.9	1.2	0.8	1.0	75	59	66
Shrink wrapping	21.7	20.2	20.9	0.9	0.4	0.6	47	34	41

The main purpose of air conditioning systems and heating is to provide a comfortable environment that does not impair the performance and health of workers (Varnam and Sutherland, 1994). As a result, bioaerosols international standards have been developed for temperature levels, air velocity and humidity (Goyer *et al*., 2001; Górny and Dutkiewicz, 2002; Dungan *et al*., 2010). There are however, few internationally recognized or agreed bioaerosol standards or occupational exposure values in food processing environments (Kang and Frank, 1989; Goyer *et al*., 2001).

The total counted microorganisms from various areas of the cottage cheese section were recorded (Fig. 2.2). From these results, it was noted that the shrink wrap area had the minimum counts taken during afternoon sessions and the packaging area had the maximum counts ranging between 25 cfu.m^{-3} and 62 cfu.m^{-3}. Samples taken in the mornings typically had higher counts than those taken in the afternoons. It was discovered that bacterial species were more prevalent in air samples than fungi (yeasts and moulds) in all sampled areas. Average bacterial counts from the processing and pasteurization areas ranged between 72 cfu.m^{-3} and 92 cfu.m^{-3}. Average fungal counts ranged between 53 cfu.m^{-3} and 58.5 cfu.m^{-3} from processing and pasteurization areas from morning samples (Fig 2.2). The American Public Health Association recommendation limits for food establishments when using air samplers are, 90 cfu.m^{-3} for aerobic counts and 180-360 cfu.m^{-3} for bacterial counts. However, from the results obtained in this study, a maximum level of 92 cfu.m^{-3} was recorded from the processing area which can increase in due course if good ventilation practices are not adhered to. As reported from a study done by Heederik *et al*., (2007), an increased bacterial loading at concentrated dairy operations has a potential to possess offsite transportation of bioaerosols.

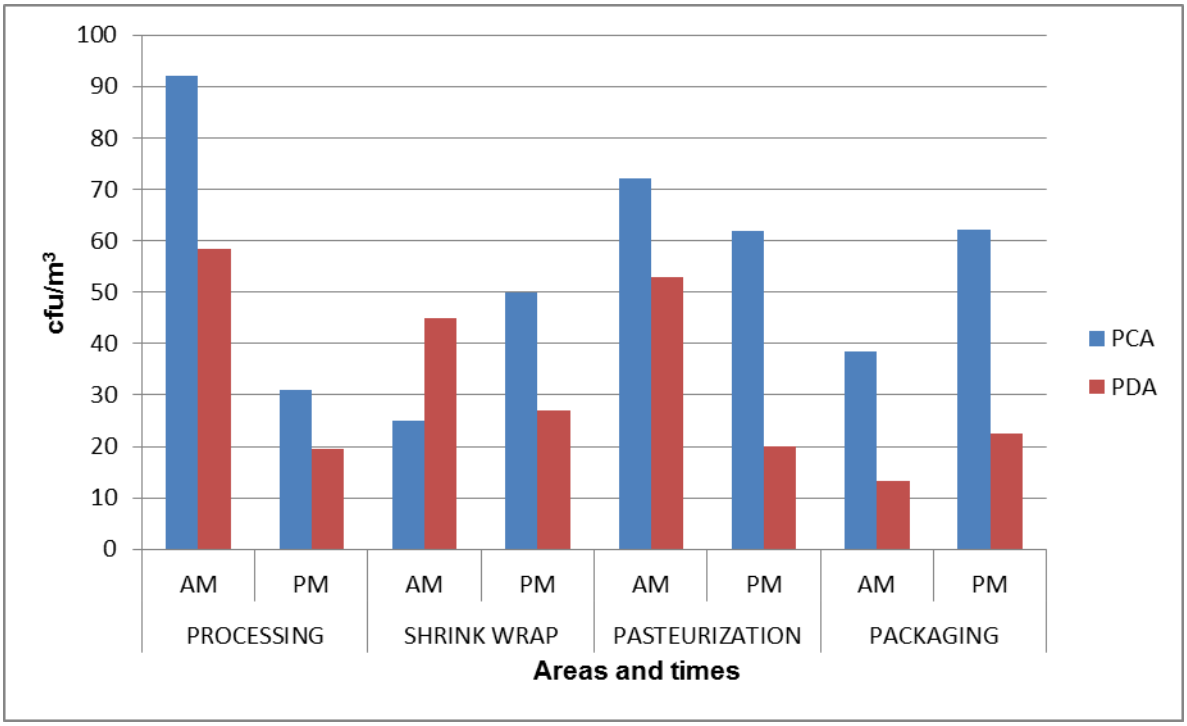

Figure 2.2: Overall bacterial and fungal counts from different sampled areas

2.4 CONCLUSION

The spoilage of cottage cheese may be attributed to many factors such as the air, water, equipment surfaces and pathogenic organisms (Dungan *et al*., 2010). The results of this study revealed the bioaerosols that potentially contaminate cottage cheese as well as the effects of extrinsic parameters such as relative humidity, wind velocity and temperature extremes. The transportation of aerosols could lead to adverse health effects in humans and livestock (Wilson *et al*., 2002; Green *et al*., 2006; Heederik *et al*., 2007). The frequency of distribution of bioaerosols within dairy industries needs thorough investigation to help prevent cross-contamination of finished products by airborne microbes.

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MICROBIAL COUNTS ON SURFACES UTILIZED DURING COTTAGE CHEESE PRODUCTION

CHAPTER 3

Abstract

The survival of microorganisms on surfaces during the production and processing of dairy products remains a challenge within dairy plants as these contaminants can lead to contamination of the final product. The aim of this study was therefore to determine the sources of microbiological contamination on surfaces utilized during the production of cottage cheese from a local dairy plant. Thirty six swabs were taken from nine different surfaces and equipment within the cottage cheese processing area. Serial dilutions of the swabs were performed within 24 hours of sample collection and quantified. Colonies were counted and Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) was used to identify isolates. Bacterial counts on the cheese contact surfaces ranged between 0.8 x 10^3 cfu.cm^{-2} and 2.8 x 10^2 cfu.cm^{-2} and fungal counts ranged between 0.5 x 10^2 cfu.cm^{-2} and 1.6 x 10^2 cfu.cm^{-2} over the entire duration of the study. Isolates identified belonged to the genera *Staphylococcus, Lactobacillus, Bacillus, Pseudomonas* and *Candida*. It was determined that cheese vat 1, cheese vat 4 and a moulded cheese container before salting were possible sources of *Staphylococcus* and *Pseudomonas* in cottage cheese. The packaging loop, cheese/milk stirrer and cheese vat 1 were found to be potential sources for yeast and mould.

Keywords: *dairy surface equipment, microbial counts, MALDI TOF MS, cottage cheese*

3.1 INTRODUCTION

Majority of foodborne outbreaks are associated with meat and dairy products that were contaminated prior to production or during production stages (Olsen *et al*., 2004; Centers for Disease Control, 2006; Eblen *et al*., 2006). Food handlers, equipment surfaces, water used during the cleaning process and ingredients used during the production stages are possible sources of contamination for dairy products (Kang and Frank, 1990; Temelli *et al*., 2006). Damp surfaces are associated with the formation of biofilms in food and dairy processing plants which assist the aggregation of microorganisms (Chmielewski and Frank, 2003; Carrasco *et al*., 2012). According to Norman and Robert (2006), all equipment within dairy processing facilities should be easily accessible and cleaned and designed for sanitizing and draining. Sanitized equipment and clean buildings are essential during production, processing and distribution to minimize contamination with harmful microorganisms.

Most dairy plants prefer to use steam, chemical sanitizers and hot water as means of sanitizing. Fogging remains an effective and easy sanitizing method for closed containers such as cheese tanks and cheese vats (Norman and Robert, 2006). Cross-contamination of cheese mainly occurs from the curd cutting knife, packaging material, floor, cheese vat, production room air, brine and cheese cloth (Temelli *et al*., 2006). Cross-contamination of these surfaces can lead to the eventual growth of pathogenic and food spoilage bacteria and yeast (Sasahara and Zottola, 1993; Doyle, 2007). Growth of spoilage causing psychrotrophic bacteria and yeast can lead to slime formation, excessive gas, off-flavours and discoloration (Rohm *et al*., 1992; Doyle, 2007; Singh and Prakash, 2008). The occurrence of yeasts in cheese can be attributed to their ability to grow at low moisture content, low pH, low storage temperatures and resistance against elevated salt concentrations (Fleet, 1990; Schlegelovà *et al*., 2010). Therefore, this study was aimed at identifying potential microbial contamination from surfaces and equipment in contact with cottage cheese during its production.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Samples were collected at four time points (one sample per surface) every Wednesday from mid-November to mid-December, within the cottage cheese section at a dairy plant in Bloemfontein. Samples were collected from: cheese vat 1, cheese vat 4, moulded cheese container before salting, moulded cheese container after salting, cheese/milk stirrer, packaging loop, cheese table surface, packaging material used during cheese production and cheese scale. All swabs were pre-wet in 0.1% sterile peptone water and an area of 3 x 3 cm was swabbed. Swabs were placed in the same diluent and placed in a cooler box within 2 hours of sampling and transferred to the laboratory. All samples were processed within 24 hours of collection.

3.2.2 Sample preparation

Serial dilutions (10^{-1} to 10^{-3}) of the samples were prepared in 0.1% (m/v) peptone buffer and 100 µL of each of the different sample dilutions were plated in duplicate onto either Plate Count Agar (PCA) (Biolab, Biolab Diagnostics, Midrand, South Africa) or Potato Dextrose Agar (PDA) (Biolab, Biolab Daignostics, Midrand, South Africa). PCA plates were incubated at 37°C for 48 hours while PDA plates were incubated at 25°C for 72 hours. Colonies were counted under a light colony counter (Vacutec, Randburg, South Africa) and expressed as cfu.cm^{-2}. Standard deviation was calculated using the formula as per the number of samples prepared for easier data caprturing.

3.2.3 Analysis using MALDI TOF MS Fingerprinting

Pure colonies selected from the PDA and PCA plates were used for MALDI TOF MS (Bruker Daltonics, Germany) identification (Van Wuijckhuijse *et al*., 2005). Where results were inconclusive or where peaks could not be detected, ethanol/formic acid extraction was performed. Isolated microorganisms (5-10 mg) were transferred to 2 ml

Eppendorf tubes and 300 µl double distilled water added (Merck, South Africa) and thoroughly mixed. Absolute ethanol (900 µl) was added to each tube and centrifuged at 13200 rpm at room temperature for 2 minutes. The residual ethanol was removed by careful pipetting and the sample left to dry at room temperature. Seventy percent formic acid (50 µl) was added to the dry pellets and mixed by vortexing, followed by the careful addition of 50 µl pure acetonitrile and centrifugation at 13200 rpm for 2 minutes. Approximately 1 µl of the supernatant was placed onto a 96 polished steel target plate (Bruker Daltonics, Germany) and allowed to dry at room temperature. Finally, each sample was overlaid with 1 µl of the HCCA matrix solution (α-Cyano-4-hydroxycinnamic acid, portioned, Sigma, USA) and air dried at room temperature.

A Microflex LT mass spectrometer (Bruker Daltonics) coupled with Flex Control software (Version 3.0) was used for the analyses of all strains. The spectrum was recorded at a laser frequency of 20 Hz, an ion source of 1V (20 kV); ion source of 2V (18.6 kV); lens voltage of 7.5 kV and a mass range of 2000 to 20 000. All the analyzed spectra were internally calibrated using *Escherichia coli* proteins as standard. The BioTyper software (Version 3.0, Bruker Daltonics, Germany) was used for the detection of raw spectra with matching standard pattern and settings; results were reported in a ranking table identified by a set of colour codes (green, yellow and red). Identity (ID) scores ranged from 0 to 3. Outcomes were expressed as proposed by MALDI-TOF biotyper (MT). The scores of <1.7 were not considered to have a reliable ID generated; whereby a score of 1.7<ID<1.9 was considered ID to genus only and a score of >1.9 was used for reliable species ID.

3. 3 RESULTS AND DISCUSSIONS

3.3.1 Microbial counts from surface swabs

Bacterial counts from all sampled surfaces ranged between 0.8 x 10^3 and 2.8 x 10^2 cfu.cm^{-2} (Table 3.1). Fungal counts ranged between 0.5 x 10^2 and 1.8 x 10^2 cfu.cm^{-2}

(Table 3.1). The highest bacterial and fungal counts were detected from cheese vat 1 (2.2×10^2 and 1.6×10^2 cfu.cm^{-2} respectively), moulded cheese container before salting (2.7×10^2 and 0.7×10^2 cfu.cm^{-2} respectively) and moulded cheese container after salting (2.8×10^2 and 0.6×10^2 cfu.cm^{-2} respectively) (Table 3.1). Cross-contamination of cheese may originate from cheese vats, cheese cloth, production room air, floor surfaces, packaging material, starter cultures, brine and curd cutting knives (Temelli *et al*., 2006). Similarly from a study done by Temelli *et al*., (2006), in this current study, bacteria and fungi were detected in cheese vat 1 and moulded cheese container before and after salting. The current results are contrary to results by Temelli *et al*. (2006), where both these studies revealed possible sources of contamination being detected from production room air as well as the cold room. Additionally, it was observed that disinfection and steam sterilization within the cottage cheese section was not fully adhered to in this study. The current practice was of concern because Legnani *et al*. (2004) indicated that contamination can result from unsterilized equipment used during the production stages and this can affect the quality of the final product.

3.3.2 MALDI TOF MS identification

A total of 32 isolates were identified using MALDI TOF MS (Table 3.2 to 3.4). Thirteen isolates were classified as Gram-positive bacteria, sixteen as Gram-negative bacteria and three as yeasts. Isolates identified using MALDI TOF MS belonged to genera of *Staphylococcus*, *Lactobacillus*, *Bacillus*, *Pseudomonas* and *Candida*. *Staphylococcus epidermidis* was isolated from cheese vat 1 and cheese vat 4 (Table 3.2). It was observed that the cottage cheese handlers use their uncovered hands inside cheese vats while cooking the cheese to assess if the curd has been cooked to a desired state. *Staphylococcus epidermidis* has been isolated from the hands and nasal swabs of beef farm workers (Wisser and Busse, 2000). Therefore, the hands of cottage cheese handlers can be regarded as a potential source of contamination. *Staphylococcus aureus* was isolated from moulded cheese container before salting and moulded cheese container after salting (Table 3.2) and has been reported to cause foodborne disease

outbreaks from dairy products such as milk, yoghurt and cheese (Giraffa *et al*., 1994; Schlegelovà *et al*., 2010).

Table 3.1: Total counts from sampled surfaces utilized during production of cottage cheese

SAMPLE NUMBERS						
SURFACE	**MEDIA**	**1**	**2**	**3**	**4**	**AVERAGE**
Cheese vat1	**PCA**	TNTC	2.0×10^2	3.1×10^2	3.9×10^2	2.2×10^2
	PDA	1.0×10^2	1.9×10^2	2.5×10^2	1.0×10^2	1.6×10^2
Cheese vat4	**PCA**	TNTC	2.3×10^2	TNTC	2.2×10^2	1.1×10^2
	PDA	1.0×10^2	0.5×10^2	0.8×10^2	0.9×10^2	0.8×10^2
Moulded cheese container before salting	**PCA**	2.3×10^2	3.2×10^2	1.7×10^2	3.5×10^2	2.7×10^2
	PDA	1.2×10^2	0.2×10^2	1.0×10^2	0.5×10^2	0.7×10^2
Moulded cheese container after salting	**PCA**	3.3×10^2	2.5×10^2	2.5×10^2	3.0×10^2	2.8×10^2
	PDA	0.5×10^2	0.3×10^2	0.7×10^2	1.0×10^2	0.6×10^2
Cheese table surface	**PCA**	0.6×10^3	TNTC	1.1×10^3	1.3×10^3	0.8×10^3
	PDA	0.2×10^3	0.7×10^3	0.4×10^3	1.1×10^3	0.6×10^3
Cheese scales	**PCA**	2.2×10^2	2.5×10^2	2.3×10^2	2.4×10^2	2.4×10^2
	PDA	0.3×10^2	0.3×10^2	0.2×10^2	0.1×10^2	0.5×10^2
Cheese/milk stirrer	**PCA**	3.0×10^3	TNTC	3.3×10^3	2.9×10^3	2.3×10^3
	PDA	1.9×10^3	1.1×10^3	1.5×10^3	1.6×10^3	1.5×10^3
Packaging material	**PCA**	2.7×10^3	2.5×10^3	TNTC	2.9×10^3	2.0×10^3
	PDA	0.4×10^3	0.8×10^3	0.6×10^3	1.3×10^3	0.8×10^3
Packaging loop	**PCA**	TNTC	2.2×10^2	2.0×10^2	2.4×10^2	1.7×10^2
	PDA	1.5×10^2	2.3×10^2	0.4×10^2	2.2×10^2	1.8×10^2

TNTC- Too numerous to count, *PDA*- Potato dextrose agar (Fungal counts), *PCA*- Plate count agar (Bacterial counts)

The remainder of the identified *Staphylococcus* species, *S. sciuri* and *S. warneri*, (Table 3.2) are also attributed to humans (Nagase *et al*., 2001; Euzéby, 2003; Le Loir *et al*., 2004). *Lactobacillus coryniformis*, *Lactobacillus lactis* and *Lactobacillus mali* were isolated from cheese vat 1, cheese vat 4 and the cheese scales (Table 3.2). *Lactobacillus coryniformis* and *Lactobacillus lactis* are used in the production of cheeses and other fermented dairy products. No human infections have been reported from consumption of food containing these bacteria (Vinderolla *et al*., 2000). *Bacillus cereus* was isolated from cheese vat 4 while *Bacillus pumilus* and *Bacillus licherniformis* were isolated from the cheese scales (Table 3.2). *Bacillus cereus* causes two types of food poisoning; the emetic and diarrheal syndromes, and a variety of local and systemic infections from contaminated and undercooked meat products, unpasteurized milk and starchy foods such as rice (Notermans *et al*., 1997). *Bacillus licheniformis* and *Bacillus pumilus* are not human pathogens or toxigenic, but have been implicated in the spoilage of dairy products (Lapage *et al*., 1992; Priest, 1993).

Gram-negative bacteria identified included *Acinetobacter junii*, *Acinetobacter baumanii* and *Acinetobacter johnsonii* (Table 3.3) which are all commonly isolated from soil and human skin. *Acinetobacter junii* was isolated from cheese table surfaces and is an emerging human pathogen (Hung *et al*., 2009; Yamamoto *et al*., 1999). *Acinetobacter baumanii* was isolated from packaging loops and causes nosocomial pneumonia (Seifert *et al*., 1993). *Brevundimonas vesicularis* and *Brevundimonas diminuta* were isolated from packaging material and both of these organisms are ubiquitous in the environment (Panasiti *et al*., 2008; Lee *et al*., 2011). *Citrobacter freundii* was isolated from cheese vat 4 and is frequently isolated from soil, water, sewage and food. It is known as an opportunistic pathogen (Wang *et al*., 2000). Another opportunistic pathogen, *Citrobacter koseri* was isolated from the cheese table surface. *Citrobacter koseri* is commonly isolated from urinary and gastrointestinal tracts of humans (Shoni, 2007; Dzeing-Ella *et al*., 2009).

Table 3.2: Gram-positive bacteria identified from sampled surfaces

ISOLATED SPECIES	ISOALTED SURFACE	SOURCE	IMPLICATIONS	REFERENCES
Bacillus cereus 4080 LBK	Cheese vat 4	Widely found in soil, water, dust, air, unpasteurized milk and 'wara' soft cheese	Emetic and diarrheal illnesses	Notermans *et al*., 1997
Bacillus licheniformis 9920004323 LBK	Cheese table surface	Soil and bird feathers	Not a human pathogen nor is it toxigenic. Food poisoning and food spoilage, contamination of dairy products	Lapage *et al*., 1992
Bacillus pumilus DSM 1794 DSM	Cheese table surface	Soil	Generally show high resistance to environmental stresses including UV light exposure	Priest, 1993
Kocuria palustries DSM 11925T DSM	Cheese table surface	Isolated from the rhizoplane of the narrow-leaved cattail	None known	El-baradei, 2007
Lactobacillus coryniformis DSM 20001T DSM	Cheese vat 1	Milk and other dairy products	None known	Vinderolla *et al*., 2000
Lactobacillus lactis DSM 20384 DSM	Cheese vat 4	Used in production of cheese and fermented dairy products	None known	Dellaglio and Sara, 1984
Lactobacillus mali DSM20444T DSM	Cheese surface scale	Isolated from fermenting musts, ciders, molasses, also found in juices	None known	Carr and Davies, 1977
Microbacterium arborescens DSM 20754T DSM	Cheese table surface	Used in some industrial processes to isomerized glucose to fructose	No reports of human infections	Godindo and Bhosle, 2009
Micrococcus luteus IMET 11249 HKJ	Cheese vat 1 and 4	Soil, dust, water, air, normal flora of mammalian skin	Colonizes the human mouth, upper respiratory tract, oropharynx	Bannerman and Peacock, 2007
Staphylococcus aureus DSM 3463 DSM	Moulded cheese container before and after salting	Dairy products, milk, cheese, yoghurt, soil, feacal matter	Associated with food borne outbreaks	Wieser and Busse, 2000
Staphylococcus epidermidis 10547 CHB	Cheese vat 1 and 4	Human skin, hands, nasal area	Endocarditis in immunocompromised patients	Wieser and Busse, 2000
Staphylococcus scuiri DSM 6671 DSM	Cheese scale	Food products of animal origin, wild animals, pets	Endorcarditis, septic shock, urinary tract infection, pelvic inflammatory disease	Lapage *et al*., 1992
Staphylococcus warneri Mb 18796_1 CHB	Cheese scale	Human skin flora	Nosocomial pathogens complicating central venous catheters	Skerman *et al*., 1980

Table 3.3: Gram-negative bacteria identified from sampled surfaces

ISOLATED SPECIES	SURFACE	SOURCE	IMPLICATIONS	REFERENCES
Acinetobacter baumanii ATCC 19606	Packaging loop	Soil, meat, vegetables, fish	Nosocomial pneumonia	Jorg-Linde *et al*., 2002
Acinetobacter junii DSM 6964T HAM	Cheese table surface	Soil	Emerging nosocomial pathogens	Yamamoto *et al*., 1999 Hung *et al*., 2009
Acinetobacter johnsoni DSM 6963T HAM	Cheese scale	Human skin, soil, dust, faecal matter	Rare cases of bloodstream infection	Seifert *et al*., 1993
Brevundimonas vesicularis DSM 7226T HAM	Packaging material	Fresh aquatic environments	Rarely isolated from clinical specimens	Panasiti *et al*., 2008
Brevundimonas diminuta DSM 7234T HAM	Packaging material	Ubiquitous in the environment	Rarely isolated from clinical specimens	Lee *et al*., 2004
Citrobacter freundii 13158_2 CHB	Cheese vat 4	Soil, water, sewage, food and the intestinal tracts of animals and humans	Is often the cause of significant opportunistic infections	Skerman *et al*., 1980
Citrobacter koseri Mu 15167_1 CHB	Cheese table surface	Usually arising from the urinary and gastrointestinal tracts	Rarely causes infection in immunocompetent patients	Shoni, 2007; Dzeing-Ella *et al*., 2009
Enterobacter cloacae 20105_2 CHB	Moulded cheese container after salting	Normal gut flora of many humans	Important cause of nosocomial infections	Hart, 2006
Gluconobacter oxydans B 544_UFL	Cheese scale	Soil, plants	None known	Yukphan *et al*., 2004
Neisseria flavescens C1 2 PGM	Packaging material	Oral cavity	Rarely causes disease	Wertlake and Williams, 1968
Pseudomonas alcaligenes DSM 50342T HAM	Packaging loop	Common soil and water inhabitant	None known	Arslan *et al*., 2011
Pseudomonas extremorientalis DSM 15824T HAM	Packaging loop	Found in drinking water reservoir	Opportunistic pathogen	Ivanova *et al*., 2002
Pseudomonas fulva 013_W30 NFI	Cheese/milk stirrer	Rice plants, grains and paddy fields	Not yet been isolated from humans as a pathogen	Seok *et al*., 2010
Pseudomonas veronii B 560 UFL	Cheese table surface	Isolated from natural springs	None	Elomari *et al*., 1996
Raoultella ornithinolytica Mb_18887 CHB	Cheese/milk stirrer	Aquatic environments, fish and insects	Human infections are exceedingly rare	Solak *et al*., 2011

Enterobacter cloacae, which forms part of the normal gut flora of many humans, was isolated from the moulded cheese container after salting and is known as an important cause of nosocomial infections (Hart, 2006). *Neisseria flavescens* and *Gluconobacter oxydans* were isolated from the cheese scale and packaging material and are normally isolated from soil and oral cavities but rarely cause disease (Wertlake and Williams, 1968). Several species of *Pseudomonas* were identified i.e. *P. alcaligenes*, *P. extremorientalis*, *P. fulva*, *P. manginali* and *P. veronii* from the packaging loop, cheese/milk stirrer and the cheese table surface respectively. The origins of *Pseudomonas* species are mainly from aquatic springs, water, soil, rice plants and grains while *Pseudomonas extremorientalis* is an opportunistic human pathogen. Three species of the genus *Candida*, namely *C. glabrata*, *C. guiliermondii* and *C. lambica* were isolated from cheese vat 1, cheese vat 4, cheese/milk stirrer and packaging material respectively (Table 3.4).

Candida glabrata is commonly isolated from human mucosal tissue and from fruit and is known to cause food spoilage (Fidel *et al*., 1999). *Candida guiliermondii* is found in buttermilk, fish and in the faeces and normal epidermal flora of animals. Although *C. guiliermondii* is rarely seen as an invasive fungal infection it does cause onychomycosis (Chen *et al*., 2010). *Candida lambica* is commonly found in dairy products, fruits and water and is associated with food spoilage particularly of dairy products. It is also known to cause bloodstream infections and arthritis in individuals suffering from alcoholism. *Candida lambica* was previously isolated from Egyptian Karish cheese and spoiled cottage cheese (Vervaeke *et al*., 2008).

Table 3.4: Yeast species identified from sampled surfaces

ISOLATED SPECIES	SURFACE	SOURCE	IMPLICATIONS	REFERENCES
Candida glabrata 31 PSB	Cheese vat 1 and 4	Human mucosal tissues, fruits	Food spoilage	Fidel *et al.,* 1999
Candida guiliermondii CBS 566 CBS	Packaging material	Normal skin and in sea water, faeces of animals, fig wasps, buttermilk, leather, fish and beer	Causes onychomycosis	Chen *et al*., 2010
Candida lamblica CBS 603 CBS	Cheese/milk stirrer	Soil, found in dairy products, fruits, water, birds and humans	Bloodstream infections. It has also been reported to be a cause of arthritis in individuals suffering from alcoholism.	Kurtzman and Fell, 2000

3.4 CONCLUSION

In this study, the prevalence of microbial contamination from equipment surfaces utilized during the production of cottage cheese was assessed. Bacterial counts from cheese vat 1, moulded cheese container before salting and moulded cheese container after salting, as well as the cheese/milk stirrer were higher than other studied areas such as cheese scales, cheese vat 4, the packaging loop, packaging material and cheese table surface. Yeast and mould counts were highest in the packaging loop, cheese/milk stirrer and cheese vat 1. Surfaces and equipment utilized during the production of dairy products were identified as potential source of contamination due to the presence of spoilage and pathogenic organisms (Schlegelovà *et al*., 2010). Contamination could be due to insufficient sterilization of equipment and surfaces remaining damp after sanitization (Orth, 1998). Several pathogens isolated from equipment surfaces used during cottage cheese production could have health implications if consumed. In order to limit the growth of these bacteria and fungi, strict health and safety protocols should be adhered to.

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INFLUENCE OF STORAGE CONDITIONS ON ORGANIC ACID PROFILES OF COTTAGE CHEESE

CHAPTER 4

ABSTRACT

Cottage cheese is a soft, mild flavoured cheese that is prone to contamination and requires low temperatures to maintain its shelf life. Organic acids within cottage cheese play a major role in influencing the organoleptic properties as well as stabilization of the microbial culture. Analyses of organic acid profiles are vital in determining the spoilage of cottage cheese and its shelf life. In this study, Ion Exchange High Performance Liquid Chromatography (HPLC) was used to identify and quantify the presence of different types of organic acids within cottage cheese. Samples of cottage cheese were either analyzed directly from the dairy plant or during storage at room temperature, 27°C and 4°C for five consecutive weeks. Changes in organic acid concentrations and types were recorded. The organic acids acetic, citric, oxalic and uric acid were confirmed to be present in the cottage cheese samples. Generally, cottage cheese collected fresh from the factory and those stored at 4°C, exhibited low organic acid content of between 0.01 $mg.ml^{-1}$ and 0.05 $mg.ml^{-1}$. Samples fresh from the dairy plant recorded a maximum concentration of oxalic acid and lactic acid of 0.054 $mg.ml^{-1}$ and 0.052 $mg.ml^{-1}$ respectively. Samples stored at room temperature 27°C recorded a maximum concentration of lactic acid of 0.11 $mg.ml^{-1}$ .After two weeks, refrigerated samples recorded a maximum concentration of oxalic and lactic acid of 0.056 $mg.ml^{-1}$ and 0.057 $mg.ml^{-1}$ respectively. Refrigeration of cottage cheese at 4°C for up to five weeks proved to be an effective way of preserving organic acids since their chromatographic profiles were similar to those of cottage cheese fresh from the production line.

Keywords: *organic acids, HPLC, cottage cheese, storage conditions*

4.1 INTRODUCTION

Cottage cheeses are soft, mildly flavoured and highly perishable cheeses that can serve as growth medium for many spoilage organisms (Rosenberg *et al*., 1994; Pereira-Dias *et al.*, 2000; Fadda *et al*., 2001; Kavas *et al*., 2006). Cottage cheese is considered an excellent source of calcium, vitamins, proteins, fat and carbohydrates (Neugebauert and Gilliland, 2005). Moreover, cottage cheese and other semi soft cheeses are vulnerable to spoilage by microorganisms such as coagulase positive *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, coliforms, *Pseudomonas* spp., yeast and moulds (Little and Knøchel, 1994; Whitley *et al*., 2000; Viljoen, 2001). Dogan and Boor (2003) revealed that soft cheeses with a pH of 5.0 to 6.5 as well as high moisture content of 50% to 80% are particularly susceptible to spoilage by proteolytic and lipolytic enzymes of the psychrotrophs and *Pseudomonas* spp. in particular.

Organic acids can result from the fermentation process in addition to being found naturally in raw milk. The presence of organic acids in cottage cheese plays a major role in influencing the organoleptic properties as well as stabilization of the microbial culture (Kosikowski and Mistry, 1999a; Fox *et al*., 2004a). Organic acids are classified as typical products for metabolism of microorganisms (Gomis, 1992). Majority of organic acids occur naturally in foods and are increasingly popular as food preservatives (Nakai and Siebert, 2003; Theron and Lues, 2007). High Performance Liquid Chromatography (HPLC) is an analytical technique used for the separation of compounds soluble in a particular solvent. Organic acids in dairy products such as whole milk, skim milk powder, cultured buttermilk, sour cream, cottage cheese, yogurt, sharp Cheddar cheese and blue cheese have been analyzed using HPLC (Marsilli *et al*., 1981). Reversed-phase (RP-HPLC) was previously used for determining the pH values of the mobile phase which affects the chromatographic behavior of organic acids (Zhao *et al*., 2001). Therefore, the main objective of this study was to quantify the organic acid profile of fresh cottage cheese during two different storage temperatures to assess changes in quality.

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

Eighteen samples of cottage cheese were collected from a dairy plant in Bloemfontein, South Africa. Twelve samples were collected once off and stored at 4°C and at room temperature (27°C) respectively. Six fresh samples were collected on a weekly basis for 6 consecutive weeks. All samples were transported to the laboratory on ice and the first 3 samples under different storage conditions were analyzed within 6 hours on arrival. Organic acids were extracted from the cottage cheese samples using the method described by Bouzas *et al*. (1993).

4.2.2 Sample preparation

Organic acid standards associated with natural milk and cheeses (acetic, butyric, citric, huppuric, formic, fumaric, isovaleric, lactic, malic, orotic, oxalic, propionic, succinic, valeric and uric acid) were obtained from Merck, Midrand, South Africa. Fresh cottage cheese samples were analyzed for organic acids consecutively for five weeks. All samples were analyzed within 6 hours of collection. Cottage cheese stored at 4°C and at room temperature (27°C), were analyzed each week of storage for five consecutive weeks.

4.2.3 High Performance Liquid Chromatography equipment and operating conditions

Organic acids in cottage cheese were separated by ion exchange HPLC using an automated system (Spectra Physics) equipped with a solvent degasser (SCM 400), quaternary gradient solvent pump (P4000), multi-auto sampler (AS1000) fitted with 50 µl loop and a spectral array UV detector (UV 3000) set at 210 ηm and 290 ηm. All separations were carried out on a Phenomenex® Rezex ROA-Organic Acid H+ (8%), 300 X 7.8mm ion exchange column at a constant temperature of 55°C. Isocratic elution

was done using 0.005 N H_2SO_4. Detection was done at 210 ηm and 290 ηm. The elution rate was at 1 ml.min^{-1} and the total run time was 30 minutes with an injection volume of 10 µl. The chromatograms of the three cottage cheese samples were compared to those of the organic acid standards. The retention times of the standard organic acids were used to confirm the presence of those standards in the cottage cheese samples (Table 4.1). Standard calibration curves of the positively identified organic acids were set up to quantify the amount of organic acids in the cottage cheese samples.

Table 4.1: Organic acids retention times and the correlation coefficients

Organic acid	Retention time	Correlation coefficient	Linear equation
Acetic acid	11.8	0.999	$7 \times 10^{-8}X - 0.0008$
Citric acid	6.8	1	$2 \times 10^{-8}X$ 0.0001
Oxalic acid	5.5	1	$2 \times 10^{-8}X$ 0.0002
Lactic acid	10.3	1	$2 \times 10^{-8}X - 0.0002$
Uric acid	11.8	0.998	$2 \times 10^{-8}X$ 0.00018
Orotic acid	6.8	1	$4 \times 10^{-7} + 0.0007$

4.3 RESULTS AND DISCUSSIONS

All 11 organic acid standards used were detected on the ion exchange chromatogram (Appendix C). A total of 7 organic acids, namely: oxalic acid, orotic acid, citric acid, lactic acid, acetic acid, fumaric acid and uric acid (Appendix D and E) were confirmed to be present in cottage cheese. A dual wavelength detection system was used because there was co-elution of citric acid and orotic acid at a retention time of 6.8 minutes. At 11.8 minutes, acetic acid, uric acid and fumaric acid were also eluted at the same time (Appendix C). Fox *et al*. (2004) illustrates that formic-, acetic-, butyric- and propionic acids are volatile, thus contributing to the aroma of cottage cheese. Orotic acid and uric acid were detected at 290 ηm, while the remainder of the organic acids were detected at 210 ηm. The systems used could not differentiate between acetic acid and fumaric acid since both of the acids were absorbed at 210 ηm and eluted at the same time.

Lactic acid eluted after 10.3 minutes and was noted as the dominant organic acid. Other organic acids reported were oxalic acid and citric acid. Fox *et al*. (2004) illustrates that the lactic acid concentration of cottage cheese ranges from 124 $mg.kg^{-1}$ to 452 $mg.kg^{-1}$, providing cottage cheese with its acidic taste. Fox *et al*. (2004) and Hugenholtz *et al*. (1995) report that in cottage cheese, acetic acid concentrations range from 11-292 $mg.kg^{-1}$, formic acid concentrations range from 23-306 $mg.kg^{-1}$ and propionic- and butyric acids occur at low concentrations of <1 $mg.kg^{-1}$. Storing cottage cheese at 4°C for one week resulted in no essential change in organic acid concentrations (Fig 4.1). The organic acid levels remained relatively low at similar or lower concentrations compared to the fresh cottage cheese (Fig 4.2). A similar organic acid profile was noted for both fresh and refrigerated cottage cheese. These results indicated that keeping cottage cheese at a temperature of 4°C is an efficient way of preserving the organic acid profile and also retaining the diacetyl flavour as observed by Fox *et al*. (2004).

Storing cottage cheese at room temperature resulted in a significant increase in lactic acid concentration (Fig 4.3). A maximum concentration of 0.12 $mg.ml^{-1}$ of lactic acid was recorded at week two. All other organic acid concentrations remained similar to those in

cottage cheese fresh from the dairy plant or stored at 4°C. This increase in lactic acid is probably due to the increased activity of lactic acid fermenting bacteria used in the production of cottage cheese due to storage at the high relative room temperature of 27°C. Cottage cheese can be stored at these temperatures not longer than 3-4 days after opening.

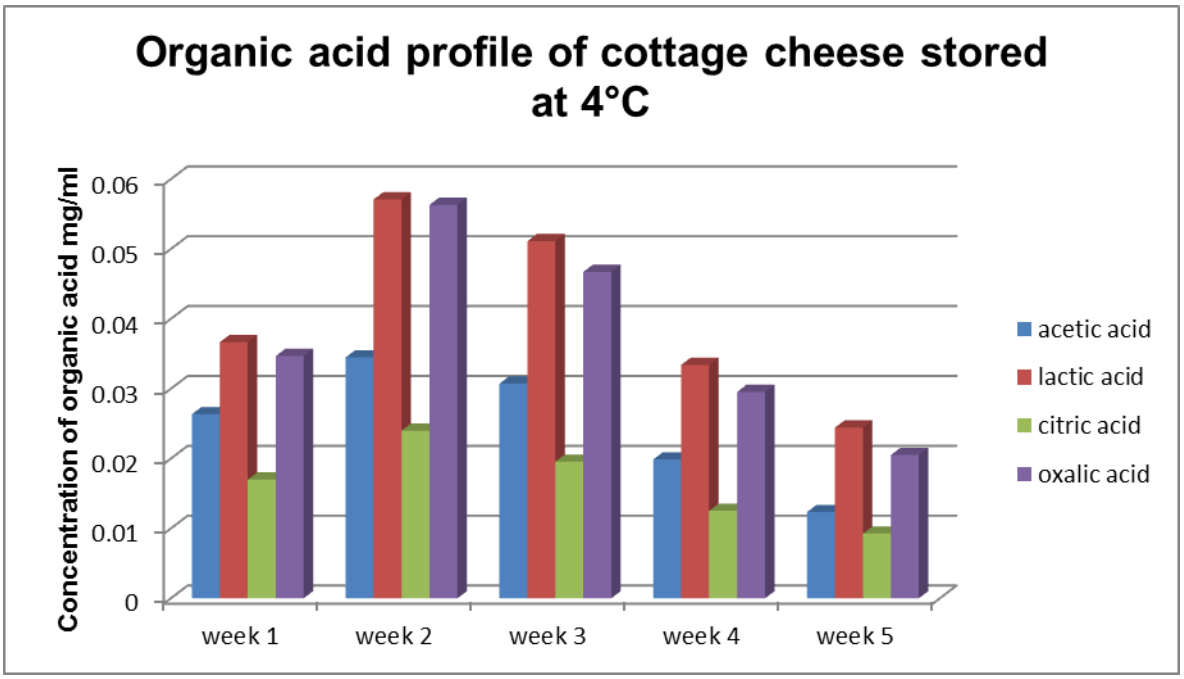

Figure 4.1: Cottage cheese stored at 4ºC.

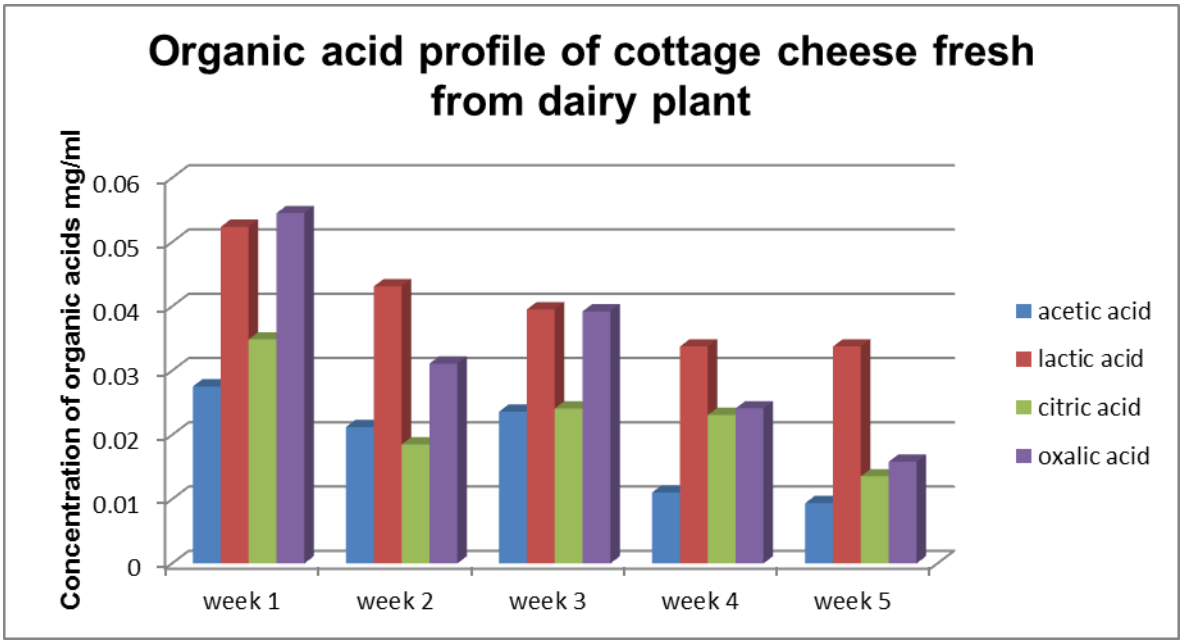

Figure 4.2: Cottage cheese fresh from dairy plant

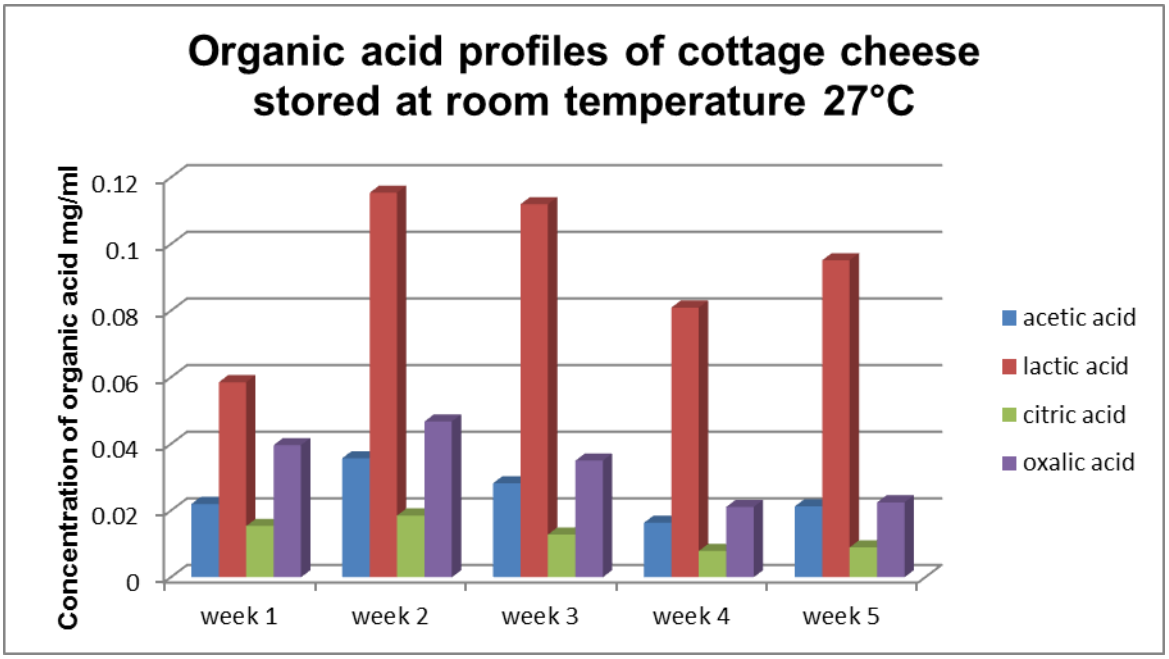

Figure 4.3: Cottage cheese stored at room temperature 27 °C

4.4 CONCLUSION

There is limited research on the organic acid profiles of cottage cheese and their concentrations under different storage conditions. Cottage cheese samples may be stored at 4°C not longer than 3-4 days and at room temperature 27 °C, cottage cheese samples may not be stored more than 1 day. Organic acid concentrations were unaffected by storage at 4°C, however, storage at room temperature 27 °C revealed an increase in lactic acid concentrations. Despite this, storage of cottage cheese at refrigeration temperatures is necessary to prevent spoilage. Further research regarding the availability and standards of organic acid profiles in cottage cheese is needed locally and internationally in order to determine the important role that organic acids play within cottage cheese and other related food products.

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SHELF LIFE STUDY AND MICROBIAL PROFILE OF COTTAGE CHEESE

CHAPTER 5

ABSTRACT

Cottage cheese is a cheese curd product with a mild flavour. Due to high water content, low fat and mild pH, cottage cheese has a short shelf life. The aim of this study was to examine the shelf life of smooth cottage cheese and the microbial load of cottage cheese from a local dairy plant in Bloemfontein, South Africa. Cottage cheese samples were collected from the dairy plant and analyzed for five consecutive weeks stored at either 4°C or 27°C. Fresh cottage cheese was also analyzed each week. For the enumeration of coliforms, yeasts and moulds, *Staphylococcus* and *Pseudomonas* species: Violet Red Bile Mug Agar, Baird-Parker Agar, Rose Bengal Chloramphenicol Agar and *Pseudomonas* Selective Agar were used respectively. pH measurements were also done on a weekly basis. Microbial counts ranged from 1.0 x 10^2 cfu.g^{-1} to 8.6 x 10^3 cfu.g^{-1} while fungal counts ranged from 1.0 x 10^3 cfu.g-^{1} to 4.0 x 10^3 cfu.g-^{1} over course of the study. However, coliforms were not detected in the cottage cheese samples analyzed. Cottage cheese samples fresh from the factory had the lowest microbial and fungal counts. The pH of the cottage cheese samples ranged between 3.04 and 4.42 under all storage conditions. From the second week, the average pH of the cottage cheese samples stored at 4°C was 4.25, which was slightly higher than the average pH of 4.15 for cottage cheese samples stored at 27°C. Cottage cheese stored at 4°C and 27°C spoiled in 3 and 2 weeks respectively. In conclusion, storing cottage cheese at 4°C is essential for prolonging its shelf life.

Keywords: *shelf life, storage conditions, spoilage microorganisms, cottage cheese*

5.1 INTRODUCTION

Variations in the microbiological, biochemical and sensorial parameters of cheese are dependent on the quality of raw materials, manufacturing protocol and hygiene status of the manufacturing plant. In general, uniformity is one of the most sought-after attributes in cheese quality due to its economic implications. The production methods used in cheese requires high temperatures to make them palatable (Mead *et al*., 1999). Due to its low water activity and high moisture content of 80%, relatively high pH of 4.5 – 4.8, cottage cheese is susceptible to spoilage and serves as a good substrate for growth of microorganisms including pathogens such as *Salmonella* spp., *Campylobacter* spp., coagulase positive staphylococci, *Listeria monocytogenes* (Kelly and O' Donnell, 1998; De Buysier *et al*., 2001; Salandra *et al*., 2008).

The shelf life of cottage cheese is typically between 21 to 28 days under refrigeration. Addition of preservatives and additives in the production of cottage cheese help to increase the shelf life and appearance (Mead *et al*., 1999). Factors such as storage temperature, pH, salt content, packaging material and storage period can influence microbial growth in the cheese (Brocklehurst and Lund, 1988; Chen and Hotchkiss, 1991). Cottage cheese spoilage microbes include coliforms and psychrotrophic *Pseudomonas* species the growth of which can lead to slimy curds, undesirable off-flavours and pigment formation (Houghtby *et al*., 1992; Jay, 2000). *Pseudomonas* species require high water activity for growth but are inhibited by pH values of 5.4 therefore cottage cheese is highly prone to *Pseudomonas* contamination (Doyle, 2007). In cottage cheese, the growth of yeasts and moulds like *Geotrichum, Penicillium, Mucor* and *Alternaria* can also result in spoilage (Zakrzewski *et al*., 1991; Jay, 2000). No previous studies have been done on the microbial proliferation and contamination of smooth cottage cheese in the production environment in South Africa. In this study microbial counts were established for cottage cheese at refrigerated storage of 4°C and at room temperature storage of 27°C. The pH was measured weekly from week 1 to 5.

5.2 MATERIALS AND METHODS

5.2.1 Microbiological analysis

Cottage cheese samples were randomly selected from the production line of a dairy processing facility in Bloemfontein, South Africa. Sampling was conducted for five consecutive weeks during the months of September and October, 2012. Immediately after each collection, samples were placed on ice and transported to the laboratory and analyzed within 24 hours.

5.2.2 Sample preparation

Cottage cheese was sampled in triplicate (125 g/sample) for five consecutive weeks and labelled as 1,2,3,4 and 5 weeks and stored in the laboratory fridge 4°C and laboratory room temperature of 27°C before analysis. Fresh cottage cheese was also collected and analyzed weekly. Aliquots of 10 g were weighed from samples and aseptically transferred to 10 mL buffered peptone water and mixed thoroughly. Serial dilutions of the samples were prepared (10^{-1} to 10^{-3}) and 1 mL of each dilution plated in duplicate. For the enumeration of coliforms, yeasts and moulds, *Pseudomonas* and related species and *Staphylococcus* and other related species, the following Agar was used respectively: Violet Red Bile Agar, Rose Bengal Chloromphenicol Agar, *Pseudomonas* Selective Agar and Baird Parker agar. All agars were purchased from Merck, South Africa. From each McCartney bottle, 1 mL of the solution was poured into the different selective media in duplicate and incubated according to manufacturer's instructions. Colonies were counted and expressed as number of colony forming units per gram of sample ($cfu.g^{-1}$).

5.2.3 pH Measurements

The pH was measured for all cottage cheese samples after 1, 2, 3, 4 and 5 weeks of storage at 4°C and 27°C respectively, using the Crison Glp 21 pH meter (Crison, South Africa).

5.3 RESULTS AND DISCUSSIONS

Total microbial counts varied between 1.0 x 10^2 cfu.g^{-1} and 8.6 x 10^2 cfu.g^{-1} represented by cottage cheese stored at 4°C, cottage cheese stored at room temperature and fresh cottage cheese from the factory (Table 5.1). Coliforms were not present in the cottage cheese samples. Similarly, no coliforms were detected in a study on Ethiopian fermented cottage cheese (Eyassu, 2013). The absence of coliforms in the cottage cheese samples may have been due to a low pH of 4.0 (Eyassu, 2013). During the five week study, total fungal counts varied between 1.1 x 10^2 cfu.g^{-1} and 3.0 x 10^2 cfu.g^{-1}. Fresh cottage cheese samples from the factory, fungal colonies were only detected during the last two weeks of the study and had the lowest counts of 1.1 x 10^2 cfu.g^{-1} to 2.9 x 10^3 cfu.g^{-1} of all samples analyzed. In week 3 cottage cheese sample stored at 4°C had slightly lower fungal counts (8.0 x 10^2 cfu.g^{-1}) than the cottage cheese sample stored at 27°C. There was no legislation or standard in South Africa that was found to help compare the amounts of organisms with.

Cottage cheese sample stored at 27°C had higher counts of *Pseudomonas* spp. reaching a peak of 8.6 x 10^2 cfu.g^{-1} in week 3. Some species of *Pseudomonas* are psychrophilic and can grow at refrigeration temperatures while others are adapted for growth at ambient temperatures as found in this study, (Lycken and Borch, 2006; Doyle, 2007). In week 3 of the study, higher microbial counts were obtained on Baird Parker Agar (BPA) plates than results obtained for week 1, 2, 4 and 5. It was also noted that *Pseudomonas* counts were higher in week 2 and 3 of the study than results obtained forweek 1, 4 and 5. Bacterial counts for cottage cheese samples from 4°C grown on

Table 5.1: Average bacterial and fungal counts from different cottage cheese samples

CHEESE SAMPLES	MEDIA	BACTERIAL AND FUNGAL COUNTS (cfu.g^{-1})				
		WEEK 1	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**
Cottage cheese stored at 4°C	BPA	1.3 x 10^2	1.1 x 10^2	5.0 x 10^3	1.4 x 10^2	1.8 x 10^2
	VRBA	0	0	0	0	0
	PSEUDO	2.7 x 10^2	5.9 x 10^2	8.0 x 10^2	3.0 x 10^2	4.1 x 10^2
	RBCA	2.0 x 10^3	3.0 x 10^3	1.6 x 10^3	0	0
Cottage cheese stored at room temperature 27°C	BPA	3.3 x 10^3	2.2 x 10^3	2.0 x 10^3	2.2 x 10^3	0
	VRBA	0	0	0	0	0
	PSEUDO	3.4 x 10^2	7.1 x 10^2	8.6 x 10^2	5.5 x 10^2	4.0 x 10^2
	RBCA	2.0 x 10^3	3.0 x 10^3	0	0	0
Fresh cottage cheese from factory	BPA	N/A	4.2 x 10^2	4.0 x 10^2	1.6 x 10^2	1.0 x 10^2
	VRBA	N/A	0	0	0	0
	PSEUDO	N/A	1.8 x 10^2	1.0 x 10^2	1.3 x10^2	2.1 x 10^2
	RBCA	N/A	0	0	2.9 x 10^3	1.1 x 10^3

BPA- Baird Parker Agar; *VRBA*- Violet Red Bile Mug Agar; *PSEUDO*- Pseudomonas Selective Agar; *RBCA*- Rose Bengal Chloromphenicol Agar, N/A – Not applicable , 0-no growth

BPA were recorded as 1.3 x 10^2 cfu.g^{-1} after the first week. At week 3 bacterial counts peaked at 5.0 x 10^3 cfu.g^{-1}, but dropped down to 1.4 x 10^2 at week 4. This could be attributed to a depleted nutrient supply or due to the presence of lactic acid.

Moreover, *Pseudomonas* counts averaged at 2.7 x 10^2 cfu.g^{-1} in week 1 and reached a peak of 8.0 x 10^3 cfu.g^{-1} in week 3. Week 4 and 5 showed a decrease in *Pseudomonas* counts with 3.0 x 10^2 cfu.g^{-1} and 4.1 x 10^2 cfu.g^{-1} respectively. The bacterial counts from this study were low compared to those seen in a study on Ethiopian fermented cottage cheese, where total bacterial counts ranged from 2.5 x 10^5 to 6.9 x 10^7 cfu.g^{-1} (Eyassu, 2013). Fungal counts for cottage cheese samples stored at 4°C averaged at 2.7 x 10^3 cfu.g^{-1} in week 1 and peaked at 3.0 x 10^2 cfu.g^{-1} in week 3. However, no growth was recorded for week 4 and week 5. A possible reason for this is that yeast species such as *Geotrichum candidum* contaminates low fat cottage cheese leading to a low diacetyl concentration after 15-19 days of storage at 4-7 °C (Antinone and Ledford, 1993). Results from cottage cheese stored at 27°C revealed that *Staphylococci* counts from BPA plates were highest at week 1 with 3.3 x 10^3 cfu.g^{-1} and decreased to 2.2 x 10^3 cfu.g^{-1} in week 4. Surprisingly no colonies grew at week 5 of the study. According to the NSW Australian Authority (2009) a coagulase positive staphylococci count of between 10^3 cfu.g^{-1} and 10^4 cfu.g^{-1} for ready to eat foods, such as cottage cheese, is considered to be unsatisfactory. The counts from *Pseudomonas* plates stored at 27°C revealed that the number of colonies increased progressively from week 2 to 5 with counts of 4.0 x 10^2 cfu.g^{-1}, 5.5 x 10^2 cfu.g^{-1}, 7.1 x 10^2 cfu.g^{-1} and 8.6 x 10^2 cfu.g^{-1} respectively. The fungal counts recorded for the first and second week were 2.0 x 10^3 cfu.g^{-1} and 3.0 x 10^3 cfu.g^{-1} respectively. No growth was recorded for week 3, 4 and 5 because there was no longer moisture content favourable for fungal growth within cottage cheese samples stored at 27°C.

Bacterial counts detected on BPA containing freshly sampled cottage cheese were 4.0 x 10^2 cfu.g^{-1} and 4.2 x 10^2 cfu.g^{-1} in week 2 and week 3 respectively. Lower counts of 1.6

x 10^3 cfu.g^{-1} and 1.0 x 10^3 cfu.g^{-1} were recorded from week 4 and 5. Bacterial counts observed from *Pseudomonas* plates were lower than counts recorded in BPA plates. Benkerroum and Sandine (1988) reported that cottage cheese samples not treated with nisin, spoiled 1 week earlier than those with nisin. *Pseudomonas*, *Enterobacter* and other spore formers survive and grow well under refrigeration (Dogan and Boor, 2003; Mayr *et al*., 2004). pH is measured in food products to detect whether they are acidic or alkaline, the pH scale ranges from 0 to 14 and most bacteria will grow best at pH 7.0. However, with a pH of below 4.0 or above 10.0, most bacteria will not grow (Brock *et al*., 1984). pH measurements of all cottage cheese samples analyzed ranged between 3.04 and 4.42 for the duration of the study (Table 5.2). From week 1, cottage cheese samples stored at 4°C measured a pH 4.42 which was marginally higher than the pH 4.33 of cottage cheese samples stored at 27°C. Cottage cheese samples stored at 4°C showed little change in pH values from week 1 to week 4 with a decrease from pH 4.22 to pH 3.80 from week 4 to 5. The same pH changes were observed from fresh cheese sampled from the factory with pH 4.30 in week 1 and pH 4.25 in week 5 of the study. The trend was also observed in the cheese samples stored at room temperature with the pH values dropping from 4.33 in week 1 to 3.04 in week 5.

Fresh cheeses such as cottage cheese and camembert cheese stored in aerobic conditions results in rapid spoilage (Gammariello *et al*., 2009). High moisture content of between 50% to 80% and low acidity of pH 5.0 to 6.5 in soft cheeses promotes the growth of spoilage bacteria from genera of *Pseudomonas, Flavobacterium* and *Alcaligenes* (Dogan and Boor, 2003). Spoilage by these bacteria results in off flavour, colour defects and sliminess in cottage cheese (Brocklehurst and Lund, 1985). There is a lack of research in South Africa with regards to shelf life and microbial load of cottage cheese. However, Boikhutso (2009) did a study on the physicochemical, microbial and sensory evaluation of desserts manufactured from cottage cheese which reports an increased pH and percentage titratable acidity due to lactic acid production. Boikhutso (2009) further revealed that addition of fruit pulps within cottage cheese desserts also increases its shelf life by 7 days.

Table 5.2: pH measurements from different cottage cheese samples

CHEESE SAMPLES	pH MEASUREMENTS				
	WEEK 1	**WEEK 2**	**WEEK 3**	**WEEK 4**	**WEEK 5**
Cottage cheese stored at 4 ºC	4. 42	4. 25	4. 30	4.22	3.80
Cottage cheese stored at 27ºC	4.33	3.80	3.65	3.06	3.04
Cottage cheese fresh from factory	4.30	4.25	4.28	4.00	4.25

5.4 CONCLUSION

Several methods have been implemented to increase the shelf life of cottage cheese, either by addition of bifidobacteria, potassium sorbate, nisin compounds or modified-atmosphere packaging (Schmidt and Bouma, 1992; Gammariello *et al*., 2009). In general, possible contamination defects of cottage cheese other than yeast and moulds were revealed such as contamination by *Listeria monocytogenes* and *Clostridium sporogenes* (Kosikowski and Brown, 1973; Viljoen, 2001; Temelli *et al*., 2006). From this study, organisms from *Pseudomonas*, *Staphylococcus* and unidentified yeasts and moulds have been identified as possible spoilage organisms, resulting in reduced shelf life of cottage cheese. Further research is needed in South Africa to identify possible contamination sources of cottage cheese and standards should be developed for microbial limitations.

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FOOD HANDLERS' BEHAVIOUR, KNOWLEDGE AND HYGIENE PRACTICES DURING THE PRODUCTION OF COTTAGE CHEESE

CHAPTER 6

ABSTRACT

Food safety has raised public concerns that may necessitate future sterilization of milk products, thus altering its marketability and taste. Dairy products such as cottage cheese do not undergo maturation, thus increasing the risk of microbial contamination that could result in off-odours, slime formation and discolouration. The aim of this study was to assess hygiene practices, behaviour and knowledge of food handlers during cottage cheese production through self-administered questionnaires and an observational checklist. Results revealed that 53.8% of food handlers washed processing equipment with warm water, a brush and detergent after every use, while the remaining 46.2% admitted to not doing so. During the production of cottage cheese, it was noted that 61.5% of food handlers used their bare hands to feel if the curd was cooked to a desired state. Food handlers had also experienced contamination of cottage cheese due to foreign matter (glass splints) and 100% of them had reported the matter to management because such incidents could be a cause for concern. Thirty eight percent of food handlers were not trained in the Hazard Analysis Critical Control Points system (HACCP) or General Manufacturing Practices (GMP), resulting in a general ignorance on matters concerning production hygiene. This study highlighted the importance of food safety training and refresher training.

Keywords: *food handlers', cottage cheese, HACCP, food safety*

6.1 INTRODUCTION

Food safety remains a concern in the South African dairy industry due to poor hygiene practices observed amongst food handlers during processing (Department of Health, South Africa 2000). Dairy industries produce milk and dairy products that are consumed worldwide and known to be implicated in foodborne illnesses therefore necessitating control measures to avoid deterioration (Jordaan, 2005; Nguz, 2005; Dairy Standard Agency, South Africa, 2006). Food poisoning outbreaks from contaminated milk and dairy products are underreported in the sub-Saharan African region making documentation difficult (Nguz, 2005; Nyabila, 2005). In South Africa incidences of foodborne diseases, especially those caused by dairy products are rarely reported. The South African Health Act (Act No.61 of 2003) and the South African National Standard Code of Practice (10049:2012), state that all foodstuffs containing dairy must be of good quality (not harmful to the consumer's health or wellbeing) in order to reach the consumer in a condition that is safe for human consumption.

South Africa has a low *per capita* consumption of milk and dairy products compared to other developing countries (International Dairy Federation, 2007). In South Africa, 800 million litres of milk is used to produce 82 000 metric tonnes of cheese per year (South African Cheese, 2012). This comprises 49% Mozzarella, Cream and Feta cheeses and 51% Cheddar and Gouda (South African Cheese, 2012). Cottage cheese is characterized as an unripened cheese with a smooth texture, high moisture content and a shortened shelf life (Kosikowski and Mistry, 1999; Eyassu, 2013). During production, contamination of cottage cheese by food handlers may be unintentional due to lack of GMP, poor personal hygiene or from processing equipment (Casalta and Montel, 2008). The Hazard Analysis Critical Control Point (HACCP) system was created to identify critical focus points to help reduce microbial contamination in food safety management. HACCP is internationally recognized and has been widely adopted in dairy industries in developed countries (Foodstuffs, Cosmetics and Disinfectants Act, 1972 (R 908:2003); Ehiri and Morris, 1996; ISO 22000:2005; SANS 10330:2007; Jevšnik *et al*., 2008; Mahmoud *et al*., 2008; Karaman, 2012).

Numerous studies have identified barriers to the successful implementation of HACCP in the food industry (Gilling *et al*., 2001; Panisello and Quantick, 2001; Roberts and Sneed, 2003; Azanza and Zamora-Luna, 2005; Bas *et al*., 2007; R 962:2012). Lack of skills and training remains the major obstacle in the successful implementation of HACCP (Karaman, 2012). Predicting the behaviour of food handlers with regards to health is not always successful (Mullan and Wong, 2009). Hence a model called the Theory of Planned Behavior was used for the improvement of actual behaviour in food industries (Conner and Sparks, 2005). This research study was aimed at assessing the knowledge, general hygiene practices and behaviour patterns of food handlers during the production stages of cottage cheese.

6.2 MATERIALS AND METHODS

6.2.1 Survey

A survey was done at the request of a dairy plant in Bloemfontein, Central South Africa between November and December 2011. Information regarding the dairy processing plant was given by one of the management personnel who works within the industry and was inclusive of the name and address of the dairy plant. An experienced Environmental Health Practitioner (EHP) visited the dairy plant at normal time intervals for inspections and to assess the dairy plantof food safety practices in order to issue a. The EHP was trained in food safety practices and in possession of Certification of Acceptability (COA) in accordance with the R 962:2012, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (54 of 1972) and related standards. The survey included structured questionnaires and an observational checklist. About 50% of the dairy population were interviewed for this study but mainly from the cottage cheese section.

6.2.2 Interviewing protocol

The interviews were conducted amongst food handlers from the cottage cheese section by 3 postgraduate M.tech students from the Central University of Technology, under the Department of Life Sciences. Permission to conduct interviews was granted (ethical clearance) by the management of the dairy plant and all confidentiality forms were

signed and adhered to according to a protocol described by Van Tonder *et al*. (2007). Each questionnaire was conducted verbally and in the interviewee's preferred language which enabled the interviewer to explain each question clearly to avoid misinterpretation.

6.2.3 Survey tools

Self-administered questionnaires were used in order to give the food handlers clarity on the purpose of the study. An observational checklist was prepared to help with the assessment of the practices during production. The questionnaire consisted of open-ended questions, questions with dichotomous "yes" or "no" responses and questions asking the food handlers to state their personal opinions on "always", "sometimes" or "never" criteria. Collection of data was done by administering the questionnaires to food handlers between 10h00 and 12h00 without disrupting their normal activities. The questionnaires focused mainly on five previewed categories which consisted of a) demographic background of food handlers, b) food handlers' general hygiene, c) their knowledge about cross-contamination, d) food handlers' attitude and, e) behavioural practices during cottage cheese production. The checklist was used to establish a richer dataset, focusing on areas of importance that could lead to contamination of cottage cheese if they were not monitored or inspected on a regular basis. The questionnaire and checklist were developed with the aid of the Hazard Analysis Critical Control Point (HACCP) system, the South African Regulations relating to Milk and Dairy Products, Regulation R 1555 (1997), the Dairy Standard Agency (2006) and the World Health Organization (WHO) manual on Five Keys to Safer Food (2006).

6.2.4 Observational checklist

The observational checklist focused on three areas of importance: building and environment, storage of cleaning chemicals and general sanitation standard levels within the dairy plant. The EHP requested copies of records for chemical and microbiological analyses in order to determine whether they had adhered to standards.

6.2.5 Statistical analysis

All the data collected from the checklists and questionnaires filled out by food handlers and management were analyzed using Microsoft Excel (2007). Average percentages as well as basic descriptive statistics were recorded as described by Van Tonder *et al*. (2007).

6.3 RESULTS AND DISCUSSIONS

6.3.1 Food handlers' demographic background

Demographic studies, as shown in Table 6.1, revealed that 53.8% of the participants were young adults of 20-35 years old, and 46.2% were above 35 years old. A study done by Conner and Norman (2005), reports that most employees in their study were young adults under the age of 35 and displayed a behaviour of not adhering to proper cleaning of manufacturing equipment. Mullan and Wong (2009) also suggests that such young adults are mostly concerned with the influence of their social normative rather than their attitude towards good initiatives on handling food. The gender distribution was 46.2% female and 53.8% male. Permanent employees accounted for 53.8% of personnel with 30.7% temporarily employed and 15.5% on contract basis. 15.5% of employees had acquired tertiary education while 53.8% had secondary education and 30.7% primary education. About 69.2% of food handlers reside in the urban area of the Bloemfontein Township, Free State Province and the rest in the rural areas around the same region. The language preference of food handlers (spoken and understood) were as follows: 46.2% Southern Sothos, 15.4% Xhosas, 15.4% Ba-Tswanas, 7.6% English and 15.4% Afrikaans. The average literacy of the food handlers consisted of 53.8% who were mostly educated and 46.2% who were disadvantaged in being educated.

Table 6.1: Demographics of the food handlers

Variable (n=13)	Frequency	Occurrence (%)
Gender		
• Males	7	53.8
• Females	6	46.2
Age ranges		
• Below 20 years	0	0
• From 20 – 35 years	7	53.8
• Above 35 years	6	46.2
Educational level		
• No formal education	0	0
• Primary education	4	30.7
• Secondary education	7	53.8
• Tertiary education	2	15.5
Average literacy		
• Disadvantaged in being educated	6	46.2
• Educated	7	53.8
Location/ settlement		
• Urban settlement	9	69.2
• Rural settlement	4	30.8
Language preference		
• English	1	7.6
• Afrikaans	2	15.4
• Xhosa	2	15.4
• Setswana	2	15.4
• Sesotho	6	46.2
Employment status		
• Permanent	7	53.8
• Temporary	4	30.7
• Contract	2	15.5

6.3.2 General hygiene status of the cottage cheese section

All food handlers agreed that the dairy plant had a satisfactory level of hygiene (Table 6.2) and that their cleaning schedule was adhered to. Food handlers had general information with regards to the hygiene measures that they had to follow and agreed thatit was appropriate. All food handlers agreed that the protocols for the cleaning of equipment and premises were suitable and that the information contained in cleaning manuals is relevant to the dairy plant and conforms to the set agreed standards or specifications as stipulated in the plant procedures. Fifty three point eight percent of the food handlers reported that they always washed the processing equipment after use with warm water, a brush and soap, and 46.2% admitted to not doing so and cleaning surfaces with warm water and detergent only. This practice could lead to cheese contamination, leading to food contamination after consumption and possible foodborne outbreaks. The role that food handlers play in foodborne outbreaks is reported by Clayton *et al*. (2002) where improper handling of food contributes to 97% of foodborne illnesses. Cheese is the dairy product most frequently reported in foodborne illness cases (Singh and Prakash, 2008). Sixty nine percent of food handlers agreed that keeping cleaning records (hand washing and premises cleaning) was important for monitoring and inspections. The remaining 31% of food handlers did not adhere to the practice of keeping cleaning records which could lead to contamination of the end products. All food handlers indicated that they were aware of the importance of washing hands before, after and during the production stages of cottage cheese.

Table 6.2: General hygiene – cleaning schedule for premises and equipment

Variable (n = 13)	Frequency	Occurrence (%)
Level hygiene status in dairy plant is appropriate		
• Appropriate	13	100
Is cleaning schedule suitable for premises		
• Appropriate	13	100
Is the information contained in cleaning manual relevant?		
• Conforms to agreed standards	13	100
Washing of equipment with warm water, brush and soap		
• Followed procedure	7	53.8
• Did not follow procedure	6	46.2
Keeping of records of inspections in hand wash procedures are important or not		
• Important	9	69.0
• Not important	4	31.0
How do you wash your hands before, after and during cottage cheese production?		
• with water and soap only		
• with warm water, soap, brush and disposable towel	13	100
Washing of equipment with warm water, brush and soap		
• Followed procedure	7	53.8
• Did not follow procedure	6	46.2
Foreign matter incidents and cuts during production (can be more than one option)		
• Experienced cuts	9	69.0
• Did not experience cuts	4	31.0
• Glass splints incidents reports	13	100

It was admitted to by 69% of food handlers that upon experiencing a cut while on duty they would stop work, report the matter to the management and cover the wound by applying moisture proof dressing. The remaining 31% had not experienced such incidences. All food handlers reported cases of contamination by foreign matter (glass splints) to management in recent years as such incidents could be cause for concern. In a study conducted by Ellis *et al*. (2010) food handlers from different food premises agreed that the four motivational factors that they had used in their study, such as intrinsic and extrinsic motivation, had a definite effect on food handlers' various performances during the procedure of handling food safely. In general, employees understood the importance of household hygiene during cottage cheese production.

6.3.3 Knowledge of participants

The knowledge and behaviour practice of food handlers regarding cottage cheese production is shown in Table 6.3. All food handlers agreed that the packaging of the cheese must be done with both plastic and foil covers. All food handlers understood the importance of cold chain maintenance and they complied with the standards stipulated for all dairy products. The regulation stating the importance of the removal of protective clothing during lunch breaks was adhered to by 53.8% of food handlers. Failure to do so might cause cross contamination if the produced cheese gets into contact with clothing harbouring pathogenic microorganisms. All food handlers understood that the wiping cloth has to comply with the requirements as stipulated within the company procedures to help reduce the spread of microorganisms. All food handlers agreed that potable water used in the cottage cheese plant complies with the standards promulgated in the South African National Water Act (No. 36 of 1998) and SANS 241: 2011.

6.3.4 and 6.3.5 Food handler's observed practices and behaviour during cottage cheese production

During the production of cottage cheese, 46.2% of food handlers always used their bare hands to feel if the curd was cooked to a desired state, whereas 38.5% always used a thermometer (Table 6.4). It was noted that all of the food handlers wash their hands before, during and after the production stages. Food handlers understood the

importance of household hygiene and the “clean as you go” process, but only 38.5% admitted to always following this practice and 23.1% never did. Even though the HACCP system has been regarded a success, limitations exist due to the difficulty in establishing realistic and non-realistic hazard points, as seen in the current study (Marais *et al*., 2007).

Food handlers’ behaviour response (Table 6.5) indicated that 53.8% of food handlers admitted to taking smoke breaks. In addition, 53.8% had attended training and workshops specifically for food safety in dairy plants and had gained new knowledge, but continued with their smoking habits which forms part of their practices, whereas 46.2% of food handlers had not attended any food safety training. Amongst the smokers, 15.4% admitted that they did not wash their hands after smoking or using the toilet. Despite the negative health implications of smoking, 53.8% of smokers complained that working in a dairy was bad for their health due to the coldstress related illnesses they had suffered. The other 46.2% of food handlers were content to work at a dairy plant. Even though the dairy plant management claims to train food handlers in the Hazard Analysis Critical Control Points system (HACCP) and General Manufacturing Practices (GMP), 30.8% of food handlers in this study had no knowledge of HACCP or GMP since most of them had not received such training.

The Turkish dairy industry had foreseen the need to establish a new law that regulates all their dairy industries to comply to and then further put an emphasis on the implementation of HACCP system specifically for dairy plants (Karaman, 2012). For good HACCP implementation, critical considerations include personnel involvement, the physical plant, the environment as well as the general hygiene practices (Ehiri and Morris, 1996). Food handlers acknowledged that the only people that received training were the supervisors and only once they had received it, would they educate the rest of the food handlers. Incomplete information dissemination could be why some of them were still ignorant on matters concerning household hygiene and food safety. Subsequently, all the food handlers surveyed would like to be taught more on HACCP and GMP as only 23.1% had any knowledge concerning them.

Table 6.3: Knowledge of participants

Knowledge of participants (n=13)	% Compliance	% Non- compliance
Cheese is packaged with plastic and foil cover	100	0
Personal Protective Equipment is taken off during lunchtime	53.8	46.2
Cold chain is maintained throughout the production stages	100	0
Is the water used inside cottage cheese plant potable?	100	0
Usage of wiping cloth conforms to the normal standards of cleaning	100	0

Table 6.4: Practices of food handlers with regard to cottage cheese production

Practices of food handlers' during cottage cheese production (n=13)	SOMETIMES	ALWAYS	NEVER
Cooking of curd to a desired state			
• Using bare hands		61.5	
• Using thermometer		38.5	
Frequency of hand wash before returning to cottage cheese production	0	100	0
How often do you practice clean as you go routine?	38.5	38.5	23.1

Table 6.5: Food handlers' behavioural observation during cottage cheese production

Food handlers' behaviour (n=13)	YES (%)	NO (%)
Does your personal behaviour include in between smoke breaks?	53.8	46.2
Do you wash your hands after toilet use and smoking?	84.6	15.4
Do you think working at dairy plant is good for your health?	46.2	53.8
Have you had any training or workshop specifically for a dairy plant?	53.8	46.2
Have you gained any new knowledge from that training?	53.8	
Did it include HACCP or GMP?	23.1	30.8
Would you like to have other training in this field for empowering your knowledge?	100	

6.3.6 Results obtained from an observational checklist

During the production processes, an observational checklist was done for capturing additional aspects that were not covered in the questionnaires (Table 6.6). It was noted that the ablution facilities, waste bins, hand wash basins, taps and locker rooms were cleaned on a daily basis using clean in progress technique. The corridors, walls, floors and light switches were cleaned twice per month. Hazard Analysis Critical Control Point system was used for cleaning of all the surfaces, locker rooms, manufacturing equipment and storage of cleaning chemicals. It was again noted that ventilation systems and the bait stations were repaired and monitored once and thrice per month respectively by a consultant. As a prerequisite, the fire extinguisher, pH meter and thermometer were calibrated and serviced once per year, three times per weeks and six times per month respectively. Safety, health and environmental inspections are done on a monthly basis to help identify the hazards and risks that food handlers might be exposed to.

Table 6.6: Results from the checklist

AREA	ACTION	HOW MANY TIMES
Ablution facilities (males and females)	Clean in progress	Daily
Waste bins	Empty bin, wash in- and outside, air-dry and replace new plastic	Daily
Hand wash basins and taps	Clean in progress	Daily
Door handles	Clean in progress	Daily
Corridor	Wash walls, floors and windows, wipe all light switches	Twice per month
Locker rooms	Separate storerooms for personal items and PPE	Daily
Surfaces	Wipe down all the working surfaces (HACCP)	Before, during and after every production stages
All manufacturing equipment	Clean in progress	Before, during and after every production stage
Storage of cleaning chemicals	Clean all cupboards in- and outside	Twice per month
Lighting and ventilation	Sufficient lighting and ventilation	Once per month fixtures
Incidents or hazards	Availability of first aid kit	Always
Fire extinguisher	Service	Once per year
Milk cooling equipment	Clean as you go	Closed system operation
Pest control	On-site bait stations	Thrice per month
Instrument check-up e.g. pH meter, thermometer, cheese scale	Instrument verification and calibration	Six times per month
SHE Reps inspections	Health and safety inspections and evaluations of hazard identification risk assessments	Monthly

6.4 CONCLUSION

Food safety should never be compromised at any level and the personnel responsible for producing safe food must adhere to the requirements stipulated in the South African standards and regulations for the quality of food (South African National Standards, Code of Practice 10049:2012). Food products need to reach the consumer in a safe and palatable manner and some food handlers often ignore the good practice of keeping food safe because of poor household hygiene (Daniels, 1998)**.** Since all the production stages of this cheese type are prone to contamination training and education of food handlers remains an important factor. It is important for food handlers to have a food safety certificate prior to working in food manufacturing (Ellis *et al*., 2010). In South Africa, however, management in most food establishments do not take such matters into consideration with most food handlers not being in possession of a food safety certificate (Du Toit, 2004).

Thus the results of this study indicate that hygiene, behaviour and knowledge practices were only followed by some food handlers. Certain aspects such as not taking off protective clothing observed during the visit could lead to cross contamination. Therefore, it is recommended in this study that every employee must be trained on the safety of food with the focus area on dairy products and cottage cheese specifically. These results have once more highlighted the significance of quality control as it aimed to identify the important factors contributing to the contamination of this highly perishable cheese.

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GENERAL DISCUSSION AND CONCLUSION

CHAPTER 7

7.1 GENERAL DISCUSSION

The manufacture of cheese is essential in conserving milk proteins and extending its shelf life. The main ingredients employed during the cheese manufacturing process include milk, salt, rennet and lactic acid bacteria (Walstra, 1993; Fox *et al*., 2004a; Rattanachaikunsopon and Phumkhachorn, 2010). Various cheese varieties include soft cheeses such as cottage cheese. Cottage cheese is a nutritious food product which is highly prone to contamination (Hermann, 1996; Gammariello *et al*., 2009). The quality and safety of cottage cheese remains a critical issue in South Africa and worldwide. Cottage cheese can be easily contaminated by pathogenic and spoilage microbes the quality of ingredients used as well as food handlers' hygiene practices during manufacturing. Information regarding the acceptability of microbial load for cottage cheese is limited (Gammariello *et al*., 2009). Therefore, the aim of this study was to assess the factors, other than pathogenic microorganisms, that cause contamination and spoilage of cottage cheese. To achieve this objective, the chapters were structured into sections that correlated to the methodology of the thesis.

Chapter 2 reports on airborne microbial contaminants (bioaerosols) which have been isolated for the first time from a South African cottage cheese factory. The environmental parameters that may contribute to the distribution and prevalence of airborne microbes within the cottage cheese production areas were also evaluated. In Chapter 3, microbial concentrations on the cottage cheese equipment surfaces were calculated in order to detect the general hygiene measures of equipment during cottage cheese production. Chapter 4 reports on the organic acid profiles of cottage cheese and their effects on required storage conditions of cottage cheese. Chapter 5 further reports on the shelf life of cottage cheese in different storage conditions. Finally, Chapter 6 reports on the behaviour, attitude and hygiene practices of food handlers within the cottage cheese section during the production of cottage cheese.

7.2 CONCLUDING REMARKS ON ALL CHAPTERS

The results obtained from Chapter 2 reveal possible bioaerosol contamination and the effects of extrinsic parameters such as relative humidity, temperature and wind velocity on the quality of cottage cheese. The air samples were collected through the aid of a SAS Super 90 surface air sampler, quantified and characterized using Matrix-Assisted Laser Desorption Ionizationation Time of Flight Mass Spectrometry (MALDI TOF MS). The results from this study reveal the possible contamination of cottage cheese by airborne microorganisms from the genera *Pseudomonas, Staphylococcus, Micrococcus, Acinetobacter, Clostridium* and *Candida* amongst others. The microbial counts within the processing, pasteurization, shrink wrap and packaging areas of the cottage cheese section were not affected by temperature, but were influenced by the wind velocity and were also within the bioaerosol ranges suggested by Kang and Frank (1989) and the American Public Health Association (Hicley *et al*., 1992). The results obtained from this experiment, reveal the common strains of spoilage and pathogenic microorganisms of which the most important were associated with dairy products, normal human skin flora, hospital environments, as well as dust which could be attributed to the localization of this dairy plant. In conclusion, the identified spoilage and pathogenic microorganisms within the cottage cheese section needs thorough investigations to help prevent cross-contamination of finished products by airborne microbes within dairy plants.

The evaluations done in Chapter 3 were aimed at assessing the microbial contamination from equipment surfaces utilized during the production of cottage cheese. Equipment surfaces such as cheese vat 1, moulded cheese container after salting, moulded cheese container before salting, cheese/milk stirrer, cheese scale, cheese vat 4, packaging loop, packaging material and cheese table surface were sampled and microorganisms quantified by the use of MALDI TOF MS. Isolates included species from the genera *Staphylococcus, Lactobacillus, Bacillus, Pseudomonas, Kocuria* and *Candida* amongst others. We further determined that cheese vat 1, cheese vat 4 and moulded cheese container before salting are possible sources of contamination of cottage cheese with *Staphylococcus* and *Pseudomonas*.

The packaging loop, cheese/milk stirrer and cheese vat 1 were found to be potential sources of contamination by yeasts. Consumption of cottage cheese contaminated by the pathogenic organisms isolated from equipment surfaces hold various health implications in humans. Therefore, applicable care measures, like practicing good hygiene, are advised and should be controlled in order to limit the growth of pathogenic microorganisms on equipment surfaces utilized during cottage cheese production.

In Chapter 4, a number of organic acids were identified in cottage cheese samples analyzed using High Performance Liquid Chromatography (HPLC). Organic acids such as lactic acid, citric acid, acetic acid and oxalic acid were quantified in cottage cheese samples stored at 27°C, 4°C and cottage cheese fresh from the production line, respectively. The results revealed that organic acid concentrations were unaffected by storage at 4°C which correlates with a study done by Fox *et al*. (2004a). However, storing cottage cheese at room temperature 27°C for this study resulted in higher concentrations of lactic acid than citric, oxalic and acetic acids. Organic acid profiles for cottage cheese samples fresh from the production line and refrigerated cottage cheese samples were similar. Naturally, cottage cheese contains a relatively high concentration of lactic acid from the curds of soured skimmed milk providing it with its acidic taste. HPLC UV chromatograms revealed a distinct lactic acid peak from cottage cheese samples. Citric acid was eluted at lower concentrations than lactic acid (Ball, 2011). Lactic, acetic and propionic acids produced as end products provides an acidic environment that is unfavourable for the growth of many spoilage and pathogenic microorganisms (Ross *et al*., 2002; Rattanachaikunsopon and Phumkhachorn, 2010). From these results, lactic acid was detected in all cottage cheese samples at different storage temperatures. Storing cottage cheese at 4°C is an efficient way of preserving organic acids within cottage cheese. The promising results from this study highlight the important role that lactic acid bacteria play in cottage cheese as a starter culture, therefore improving its safety and quality. Further research is needed pertaining to the availability of standards for organic acid profiles of cottage cheese.

In Chapter 5, a shelf life study and microbial profiles of cottage cheese stored at 4°C, 27°C and fresh from the dairy plant were evaluated. Different agars were used: Baird Parker agar (BPA) (for the enumeration of *Staphylococcus* and other related species), Rose Bengal Chloromphenicol Agar (RBCA) (for the enumeration of yeasts and moulds), Violet Red Bile Agar (VRBA) (for the enumeration of coliforms) and *Pseudomonas* selective agar (for the enumeration of *Pseudomonas* and related species). pH measurements of different cottage cheese samples were also made for five consecutive weeks. Total microbial counts (*Pseudomonas* and *Staphylococcus* agars) from cottage cheese samples stored at 27°C were higher than other cottage cheese samples. Fungal growth was observed only at week 4 and week 5 in cottage cheese sampled fresh from the production line. However, no growth was detected on VRBA plates, suggesting that coliforms were not present. The pH of samples stored under different conditions ranged between 3.04 and 4.42, possibly allowing survival of spoilage and pathogenic microorganisms. Spoilage of cottage cheese samples stored at 4°C occurred in week 3 and spoilage of cottage cheese stored at 27°C occurred in week 2. Therefore, it was concluded that storing cottage cheese at 4°C is essential in prolonging its shelf life.

In Chapter 6, a questionnaire and an observational checklist were performed in order to determine and assess the hygiene status, knowledge, behaviour and attitude of food handlers during cottage cheese production. A total of 13 food handlers working in cottage cheese section of the dairy plant were surveyed. About 53.8% of food handlers are permanently employed, with the other 30.2% and 16% on temporary and contract employment, respectively. The majority of food handlers (53.8%) acquired secondary education and only 16% acquired tertiary education. All the food handlers surveyed reported that they would like to be taught more about HACCP and GMP's because 30.8% of food handlers did not have any knowledge concerning these practices. It was further revealed that the supervisors were the ones sent for training and workshops on HACCP and GMP's and upon their return was required to convey this knowledge to the food handlers. This kind of knowledge transfer could lead to an information gap due to

incomplete information dissemination. The survey showed that 30% of food handlers did not conform to the good manufacturing practices of cleaning manufacturing equipment after use and were ignorant on matters concerning household hygiene and food safety. Such negligence could lead to cottage cheese contamination by spoilage and pathogenic bacteria. Results from the observational checklist suggest that safety, health and environmental inspections are done on a monthly basis to help identify the hazards and risks that food handlers might be exposed to.

It was finally concluded from the present study, that the distribution and transportation of bioaerosols with pathogenic and spoilage microbial contaminants, insufficient sterilization of equipment surfaces, certain organic acid profiles, storage conditions, and poor hygiene practices by food handlers potentially contribute to the spoilage of cottage cheese. In brief, there were links from all the studied research chapters which contributed mainly on the spoilage and deterioration of shelf life of cottage cheese from bioaerosols, microbial contaminants, organic acids, food handlers behaviour during production stages as well as the potential limitations on cottage cheese shelf life.

7.3 RECOMMENDATIONS

From the results of this study, it is advised that food handlers receive training prior to working in the dairy industry. This would equip the food handlers with the knowledge to keep them informed on new techniques, which would also reduce the prevalence of poor hygiene practices during the production of cottage cheese. The concentration of pathogenic organisms could also be reduced if food handlers adhered to good manufacturing practices like using thermometers in stead of bare hands to feel if cheese curd is cooked to a desired state. Ventilation systems used within the cottage cheese section (in particular pasteurization area) should be monitored, maintained and serviced on a regular basis to check for compliance. This would also help to limit the distribution of bioaerosols containing pathogenic and spoilage microorganisms.

7.4 FUTURE RESEARCH

- Opportunities exist to do sensory analyses on different South African cottage cheese varieties.
- To compile and make available the agreed standards on organic acid profiles and acceptable microbial levels of the organisms highlighted in this study within cottage cheese nationally and internationally.
- To investigate the role of biofilms from equipment surfaces utilized during the production of cottage cheese and to increase awareness with regards to their impacts on the quality of the final product.

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APPENDICES

APPENDIX A

QUESTIONNAIRE USED FOR COLLECTING DATA FOR CHAPTER 6

Title: **The extrinsic and intrinsic parameters on the quality of cottage cheese**

INTRODUCTION

The outline of the questionnaire will consist of about 5 Sections A-E:

- This questionnaire is meant to be answered by both the employers and employees from the specified dairy plant.
- The collected information will be handled and managed in a strictly confidential manner.
- The correct answer will be detected by a cross [X]

QUESTIONNAIRE

Section A: **Food handlers' demographical background**

1. **Age ranges for both genders** Male ☐ Female ☐

Below 20 yrs	From 20-35yrs	Above 35yrs

2. **Language preference**

English ☐
Sotho ☐
Xhosa ☐
Tswana ☐

3. **Level of education of the participants?**

No formal education ☐
Primary school ☐
Secondary school ☐
Tertiary education ☐

4. **Average literacy of the participants**

Illiterate ☐
Literate ☐

☐

Educated

5. **Location of the participants**

Urban settlement ☐

Rural settlement ☐

6. **Employment type:**

Permanent ☐

Temporary ☐

Contract ☐

Section B: **General hygiene- cleaning schedule for premises and equipment**

7. **Do you think the level of hygiene status in the dairy plant is appropriate?**

Appropriate ☐

Non-appropriate ☐

8. **Do you think the cleaning schedule is suitable for the premises adopted for it?**

Appropriate ☐

Non-appropriate ☐

9. **In your opinion do you view the information contained in cleaning manual to be relevant?**

Conforms to agreed standards ☐

Non- conformance ☐

10. **Do you think that records of inspections in hand washing procedures are important?**

Important ☐

Not important ☐

11. **Washing of equipment with warm water, brush and soap**

Followed procedure ☐

Did not follow procedure ☐

12. **How do you wash your hands?**

With water and soap only during production	With warm water, soap, a brush and a disposable towel before, after and during production stages

13. **Have you experienced any cut from the equipment used while cleaning?**

Experienced cuts ☐

Did not experience cuts ☐

Glass splints incidents reports ☐

Section C: **Knowledge and practices of food handlers with regard to cottage cheese production**

14. **Cheese is packaged with plastic and foil cover**

Yes ☐

No ☐

15. **PPE is taken off during lunchtime**

Yes ☐

No ☐

16. **Cold chain is maintained throughout the production stages**

Compliance ☐

Non-compliance ☐

17. **Is the water used in the cottage cheese plant potable?**

Compliance ☐

Non-compliance ☐

18. **Usage of the wiping cloth conforms to the normal standards of cleaning**

Compliance ☐

Non-compliance ☐

Section D: **Attitude of food handlers with regard to cottage cheese production**

19. **Cooking of curd to a desired state**

- Using bare hands ☐ Sometimes ☐ Always ☐ Never
- Using thermometer ☐ Sometimes ☐ Always ☐ Never

20. **Frequency of hand washes before returning to cottage cheese production area**

Sometimes ☐

Always ☐

Never ☐

21. **How often do you practise “clean as you go” routine?**

Sometimes ☐

Always ☐

Never ☐

Section E: **Food handlers’ behavioural observation during cottage cheese production**

22. **Does your personal behaviour include in between smoking breaks?**

Yes ☐

No ☐

23. **Do you wash your hands after smoking and after toilet use?**

Yes ☐

No ☐

24. **Do you think working at a dairy plant is good for your health?**

Yes ☐

No ☐

25. **Have you had any training or workshop specifically for a dairy plant?**

Yes ☐

No ☐

26. **Have you gained any new knowledge from that training?**

Yes ☐

No ☐

27. **Did it include HACCP or GMP?**

Yes ☐

No ☐

28. **Would you like to have other training in this field for empowering your knowledge?**

Yes ☐

No ☐

END OF SURVEY, THANK YOU!

APPENDIX B

OBSERVATIONAL CHECKLIST

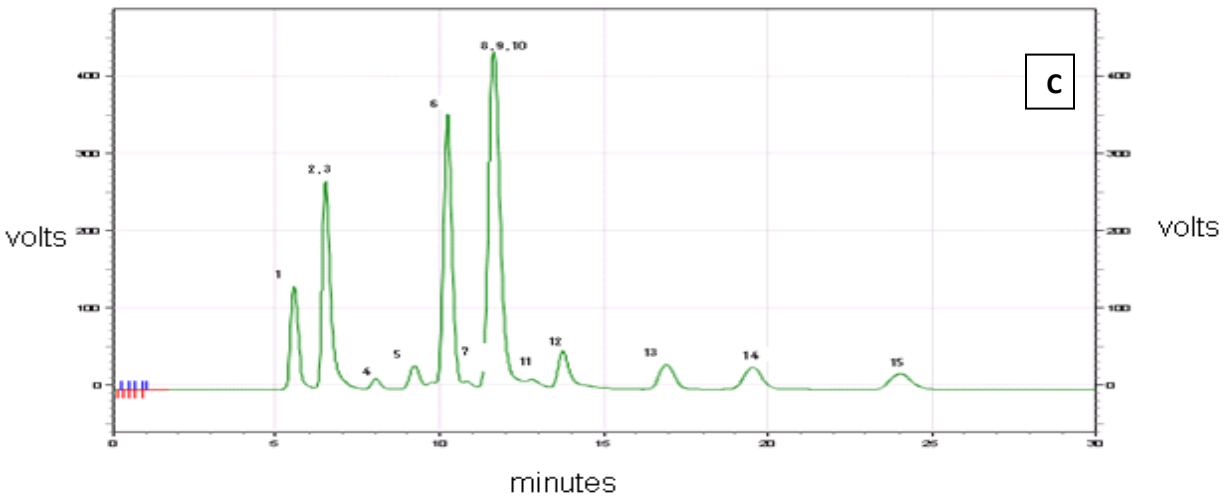
Title: The influence of the extrinsic and intrinsic parameters on the quality of cottage cheese

AREA	ACTION	HOW MANY TIMES	YES	NO	COMMENTS
Ablution facilities (males and females)	Clean in progress				
Waste bins	Empty bin, wash in-and outside, air-dry and replace new plastic				
Hand wash basins and taps	Clean in progress				
Door handles	Clean in progress				
Corridors	Wash walls and floors, windows and wipe all light switches				
Locker rooms	Separate storerooms for personal items and PPE				
Surfaces	Wipe down all the working surfaces (HACCP)				
All manufacturing equipment	Clean in progress				
Storage of cleaning	Clean all cupboards				

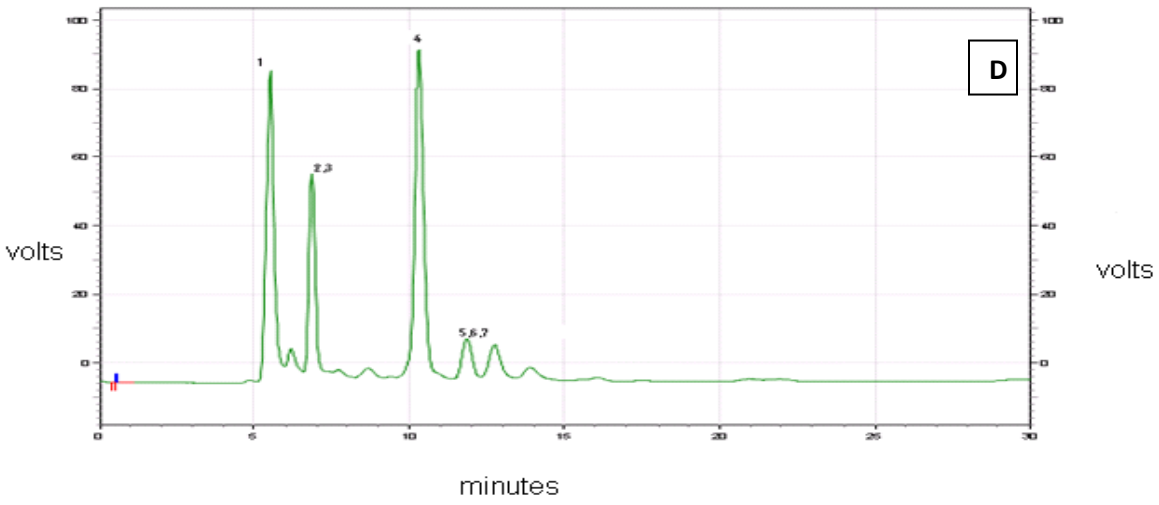
chemicals	inside and outside				
Lighting and ventilation	Sufficient lighting and ventilation				
Incidents or hazards	Availability of first aid kit				
Fire extinguisher	Service				
Milk cooling equipment	Clean as you go				
Accredited certificates	Placed on walls to check compliance				
Pest control	On-site bait stations				
Instrument check-up e.g. pH, thermometer, cheese scale	Instrument verification and calibration				
Safety, health and environmental inspections	Reports on risks and hazards				

APPENDIX C, D & E

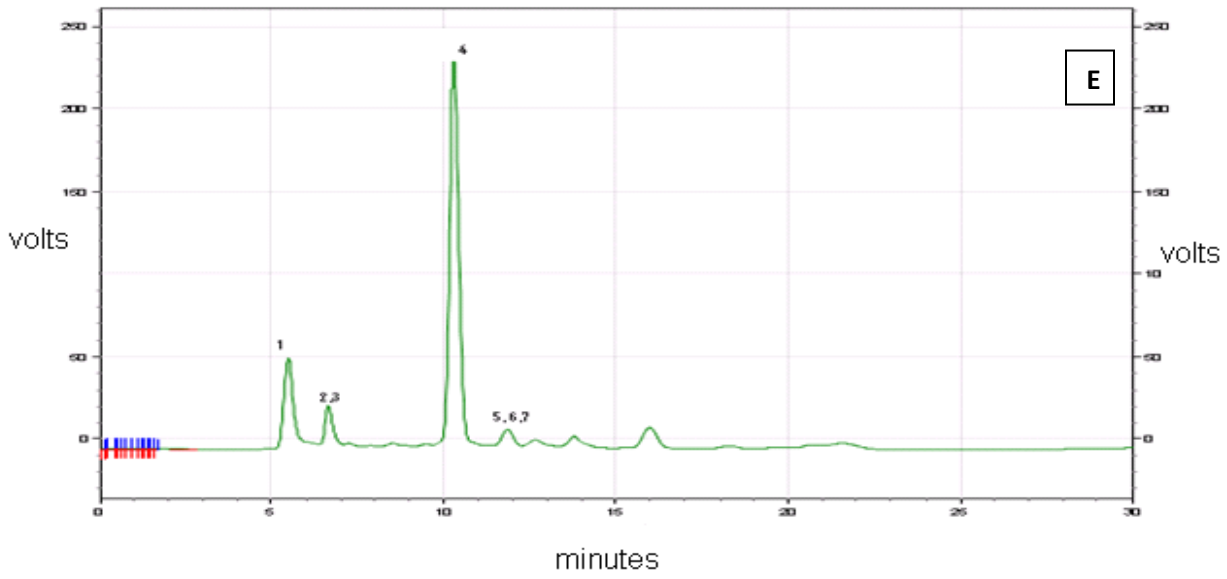
CHROMATOGRAM FIGURES USED FOR DATA IN CHAPTER 4



Chromatogram showing the mixture of organic acid standards detected at 210 ηm and 290 ηm, 1. Oxalic acid; 2. Orotic acid a; 3. Citric acid; 4. Malic acid; 5. Succinic acid; 6. Lactic acid; 7. Formic acid; 8. Acetic acid; 9. Fumaric acid; 10.Uric acid; 11. Propionic acid; 12. Huppuric acid; 13. N-Butyric acid; 14. Iso-valeric acid; 15. Valeric acid.



Chromatogram of cottage cheese fresh from the factory on the first week of analysis detected at a wavelength at 210 and 290 ηm: 1. Oxalic acid; 2. Orotic acid; 3. Citric acid; 4. Latic acid; 5. Acetic acid; 6. Fumaric acid; 7. Uric acid



Chromatogram of cottage cheese kept for one week at room temperature in the last week of analysis detected at a wavelength of 210 and 290 ηm: 1. Oxalic acid; 2. Orotic acid; 3. Citric acid; 4. Lactic acid; 5. Acetic acid; 6. Fumaric acid; 7. Uric acid