THE INFLUENCE OF AEROSOLIZED MICROORGANISMS ON THE SAFETY AND QUALITY OF FORTIFIED BISCUITS

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DECLARATION OF INDEPENDENT WORK

I, HERBERT MALISE NOE, 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 fulfillment of the requirements for the attainment of any qualification.

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SIGNATURE OF STUDENT DATE

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SUMMARY

THE INFLUENCE OF AEROSOLISED MICROORGANISMS ON THE SAFETY AND QUALITY OF FORTIFIED BISCUITS

As the concentration of dust has been shown to be proportional to seasonal change in the Free State Province of South Africa, one might expect the prevalence of associated microorganisms to follow the same pattern. The presence of dust is also associated with an aerosolised microbial population that gets blown into almost any unsealed environment including food storage facilities at schools. In addition, facility design and storage practices at these schools are under-developed and could subsequently lead to the contamination of stored food by dust, insects and rainwater. The foods in question include fortified biscuits that are intended for malnourished, and in several cases immuno-compromised, children who are susceptible to opportunistic pathogens. Therefore this study aimed to determine the impact of facility design on the level and distribution of viable airborne microorganisms (fungi and bacteria) in the storage rooms and the outdoor environment at both rural (higher dust exposure) and urban schools. Besides the pathogenicity of these organisms, their ability to degrade the sugars (major fortifying agent) in the mentioned biscuits was also established. The results showed the presence of *Escherichia coli*, which signifies faecal contamination and could be attributed to the lack of toilet facilities in the schools, especially in rural areas. Although *Staphylococcus* sp. is normally related to poor personal hygiene practices, these organisms were also isolated from the air of the storerooms and school premises. The presence of moulds and

airborne microorganisms was attributed to unfavourable environmental conditions as well as crowding in the classrooms. The microbial contamination originally present on the fortified biscuits or originating from the air further caused deterioration in the quality of the food. The fungi present in the air (identified species) cause respiratory problems when inhaled by children as they are opportunistic pathogens. It is further evident that a change of season corresponded to a general change in bioaerosol composition, such as the increased presence of dust during the winter months. It was further concluded that schools situated in different environments (urban/rural) should have storerooms that address the various environmental factors influencing bioaerosols. This would impact not only directly on the health of children in terms of their exposure to possible allergens, but also indirectly through the food that they consume as part of the feeding programme.

OPSOMMING

DIE INVLOED VAN LUGGEDRAAGDE MIKRO-ORGANISMES OP DIE VEILIGHEID EN KWALITEIT VAN DIEETAANVULLINGSBESKUITJIES

Dit is bewys dat die luggedraagde stofkonsentrasie eweredig is aan die seisonale veranderings in die Vrystaat Provinsie van Suid Afrika en dit kan verwag word dat die voorkoms van geassosieerde mikro-organismes dieselfde patroon sal volg. Die teenwoordigheid van stof word ook geassosieer met luggedraagde mikro-organisme populasies wat na enige onverseelde omgewing versprei kan word, wat voedselstoorgebiede van skole insluit. Die ontwerp van hierdie fasiliteite, asook die berginsprosedures by hierdie skole, is onderontwikkel en kan lei tot die besmetting van gebergde voedsel deur stof, insekte en reënwater. Hierdie voedsel sluit dieëtaanvullingsbeskuitjies in wat voorsien word aan kinders wat ondervoed en immunogekompromiseerd is, en dus vatbaar is vir patogene. Die doel van hierdie studie is dus om die invloed van fasiliteitsontwerp op vlakke van lewende luggedraagde mikro-organismes (fungi en bakterieë) in die bergingsgebiede en buitelugomgewings by beide landelike (hoër stof blootstelling) en stedelike skole, vas te stel. Hierdie organismes is patogenies, en hulle vermoë om suikers af te breek (die grootste aanvulling in die beskuitjies) is ook vasgestel. Die resultate het aangedui dat *Escherichia coli,* wat indikatief is van fekale kontaminasie, teenwoordig was. Dit is moontlik as gevolg van die gebrek aan sanitêre geriewe by skole, veral in die landelike gebiede. Hoewel *Staphylococcus* sp. normaalweg geassosieer word met swak persoonlike

higiëne, is hierdie organismes ook uit die lug van die stoorkamers en skoolterreine geïsoleer. Die teenwoordigheid van skimmel en luggedraagde mikro-organismes is veroorsaak deur ongunstige omgewingstoestande, sowel as die oorvol klaskamers. Die mikrobiese kontaminasie wat oorspronklik in die dieetaanvullingsbeskuitjies teenwoordig was, of wat vanuit die lug afkomstig is, veroorsaak degradering in die kwaliteit van die voedsel. Die fungi wat in die lug teenwoordig was (spesies geïdentifiseer) kan respiratoriese probleme veroorsaak wanneer dit deur kinders ingeasem word. Seisonale veranderinge stem ooreen met die algemene veranderinge in die biolugsamestelling, soos byvoorbeeld die toename van stof tydens die wintermaande. Die afleiding word gemaak dat skole in die verskillende gebiede (landelik/stedelik) bergingskamers benodig wat hierdie omgewingsfaktore aanspreek. Dit sal die gesondheid van die kinders direk beïnvloed, sowel as indirek deur die voesel wat tydens die voedingsprogram ingeneem word.

CONTENTS

		Pages
LIST OF FIGURES		**11**
LIST OF TABLES		**13**
Chapter 1:	**Introduction**	**14**
1.1	**Introductory remarks**	**14**
1.2	**Characteristics of food spoilage microbiota**	**14**
1.3	**Bioaerosols within the PSNP**	**16**
1.3.1	Inhalation	**19**
1.3.2	Contact	**20**
1.3.3	Ingestion	**20**
1.4	**Fortified biscuit storage**	**20**
1.5	**Rationale**	**26**
1.5.1	Aims and objectives	**27**
1.6	**References**	**28**
Chapter 2:	**The influence of season-associated extrinsic factors, geographical localisation and facility design on the distribution of typical airborne indicator organisms in schools participating in a feeding programme**	**34**
2.1	**Abstract**	**34**
2.2	**Introduction**	**36**

2.3	**Materials and methods**	**39**
2.3.1	Sample selection	**39**
2.3.2	Air sampling procedure	**39**
2.3.3	Facility design	**40**
2.3.4	Quantification of extrinsic factors	**40**
2.4	**Results and discussion**	**41**
2.4.1	Summary of the facility design	**41**
2.4.2	Season-associated extrinsic factors	**41**
2.4.3	Geographical localisation and airborne indicator organism presence	**44**
2.5	**Conclusion**	**48**
2.6	**References**	**50**
Chapter 3:	**Sucrose breakdown associated with Staphylococcal growth on fortified biscuits**	**54**
3.1	**Abstract**	**53**
3.2	**Introduction**	**56**
3.3	**Materials and methods**	**58**
3.3.1	Sampling site and procedure	**58**
3.3.2	Sample preparation and bacterial analysis	**58**
	Total Viable Counts	**58**
	Escherichia coli	**59**
	Staphylococcus sp.	**59**
	Aerobic and anaerobic plate counts	**59**

3.3.3	Sugar depletion by isolated *Staphylococcus* sp.	**59**
3.4	**Sugar analysis**	**60**
3.4.1	Reagents and standards	**60**
3.4.2	Sample preparation	**60**
3.4.3	Instrumentation and chromatographic conditions	**60**
3.5	**Results and discussion**	**61**
3.6	**Conclusion**	**65**
3.7	**References**	**67**
Chapter 4:	**Characterisation of airborne fungi isolated at schools participating in a feeding programme in South Africa**	**71**
4.1	**Abstract**	**71**
4.2	**Introduction**	**73**
4.3	**Materials and methods**	**74**
4.3.1	Air sampling procedure	**75**
4.3.2	Fungal identification	**75**
4.3.3	Environmental factors	**76**
4.4	**Results and discussion**	**76**
4.5	**Conclusion**	**79**
4.6	**References**	**80**
Chapter 5:	**General Conclusions**	**85**
5.1	**Background**	**85**
5.2	**Chapters 1-4**	**87**

5.3	**Recommendations**	**90**
5.4	**Future research**	**91**
5.5	**References**	**92**
	Appendix A	**93**
	Figures	
	Appendix B	**96**
	Checklist	

List of Figures

Pages

Figure 1.1	Crowded classroom in a primary school	19
Figure 1.2	Fortified biscuits served to children in the primary school nutrition programme (PSNP)	21
Figure 1.3	Overcrowded storeroom utilised for the storage of fortified biscuits	22
Figure 1.4	Fortified biscuit box stored open in a storeroom of a typical primary school partaking in the PSNP	23
Figure 1.5	Broken window in a primary school taking part in PSNP	24
Figure 1.6	Cracked and moisture-damaged wall in a storage room of a primary school partaking in a feeding programme	24
Figure 2.1	Environmental factors quantified during the dry (A) and wet (B) seasons within (α) and outside (β) storage facilities of primary schools participating in a feeding programme situated in both a rural (i, n=35) and urban (ii, n=15) environment	43
Figure 2.2	The average culturable airborne microorganisms isolated from breathable air in a primary schools (located in rural (i) and urban (ii) areas) participating in a feeding programme in Central of South Africa during both the dry (A) and wet seasons (B) in storage rooms (a) and the outdoor	46

environment (b)

Figure 3.1 Distribution of microorganisms during the dry and wet seasons. (1) Total Viable Counts (TVC), (2) *E. coli*, (3) *Staphylococcus* sp. and (4) Aerobic spore formers 62

Figure 3.2 The concentration of sucrose together with microorganisms in fortified biscuits at different time intervals 64

Figure 5.1 The SAS Super 90 microbial air sampler during sampling in the primary school storeroom 94

Figure 5.2 Fungal colonies on the Potato Dextrose Agar 94

Figure 5.3 Inspection checklist used to conduct check-up in a storage facility 95

Figure 5.4 Fortified biscuits sample collection 95

List of Tables

		Pages
Table 1.1	Bacteria and fungi previously isolated from African dust	25
Table 2.1	The storage facility design and practices in schools (rural and urban) participating in a feeding program in South Africa	42
Table 4.1	Airborne fungal genera isolated from primary schools that participate in a feeding programme in Central South Africa during both the dry and wet seasons inside and outside their storerooms	77

Chapter 1

Introduction

1.1. Introductory remarks

The Primary School Nutrition Program (PSNP), a school-feeding scheme, was introduced nationwide in South Africa in 1994, following President Nelson Mandela's announcement in his State of the Nation Address on 24 May 1994 that such a scheme would be implemented in every primary school where a need was identified (Kloka, 2003). Within the PSNP, food items are delivered to schools by private contractors and further prepared on the school premises by volunteers from the local community. The items vary from province to province and include, amongst other things, products such as fortified biscuits and protein-enriched drinks. Several items presented through the PSNP necessitate substantial handling and preparation. With metro, urban, peri-urban and rural schools participating, community volunteers apply food preparation practices primarily dictated by facility design, available financial resources and self-acquired food preparation skills.

1.2. Characteristics of food spoilage microbiota

As is the case in all commercial food production systems, ensuring the microbial safety and shelf life of foods served in the PSNP depends on reducing microbial contamination from the initial level, preventing or limiting the rate of microbial growth, or destroying microbial populations. With many foods, these strategies have been practised successfully for thousands of years. However,

microorganisms have been occupying on the earth for approximately three and a half billion years and have captured almost every imaginable habitat (McMeekin *et al*., 1997). In fact, microbial growth is only prohibited when liquid water is absent or conditions are so extreme that rapid denaturation of proteins outpaces their rate of replacement. The major characteristics that fortify the success of microbial contaminants are their small size and ease of dispersal, physiologic diversity, and tolerance of extreme conditions (Schelgel and Jannasch, 1992).

Most studies in food microbiology are concerned with the growth of microbial pathogens, although in many foodstuffs, such as fortified biscuits, the survival characteristics of the population also need to be considered. The prolonged existence of microbial spores and their resistance to harsh conditions are well documented (Gock *et al*., 2002). However, the ability of vegetative cells to resist stressful conditions is increasingly recognised as an important attribute (Archer, 1996). Consideration also needs to be given to relatively slow-growing populations in various conditions, for instance when the shelf life of a product is extended by control of rapidly growing spoilage organisms. The behaviour of foodborne microorganisms, be it the growth or death of microbial populations, is based on the time of exposure to environmental factors affecting population development. For example, equivalent destruction of bacteria in milk are achieved by low temperature-long time pasteurisation (60℃/30 min), and high temperature-short time pasteurization (72℃/15 sec). When populations are in the biokinetic range, the rate at which they grow is determined by factors such as temperature, water availability, and pH applied in food preservation procedures.

The extent of microbial growth is a function of the time the population is exposed to combinations of intrinsic food properties (e.g. salt concentration and acidity) and extrinsic storage conditions (e.g. temperature, relative humidity and gaseous atmosphere) (McMeekin *et al.*, 1997).

Different factors assume control in different foods and preservation strategies. In many foods, the full preservation potential of a single property is restricted because of considerations related to the esthetic, organoleptic and nutritional properties of the product. However, several properties or conditions may be combined to provide a desired level of stability (Leistner, 1992). In situations where the preservation approach is designed to slow the rate of population growth, the effect will always be increased by storage temperature. Temperature control in processing, distribution, and storage is essential to ensure the adequate shelf life and safety of foods. Modern technologies, including modified atmosphere packaging and sophisticated products such as sous-vide meals and fortified biscuits, do not require strict temperature control. Indeed, the requirement for observation increases with increased shelf life and the possibility of growth of psychrotrophic and osmophylic pathogens over an extended period (Beales, 2004).

1.3. Bioaerosols within the PSNP

Venter and co-workers (2003) described the microbial hazards associated with the PSNP, in addition to their possible sources, and mentioned that sources of microorganisms such as bioaerosols and their ability to proliferate on food should

also be investigated. In addition, several reports have shown associations between dust-related microbiota and a variety of health outcomes (Dutkiewicz *et al.*, 2002; Toivola, *et al.*, 2002). Microbial exposures in schools are of particular interest because of the age and susceptibility of the occupants and the possibility of long-term health outcomes such as the development of sensitivities, asthma (Taskinen *et al.*, 1997) or other effects attributed to mycotoxin and endotoxin exposures (Dales *et al.*, 1998; Savilahti *et al.*, 2000).

Despite the possibility of adverse health effects due to exposure to microbial products, no assessment of the influence of aerosolised microorganisms on the safety and quality of food stored and served in school feeding programmes has been done. In part this is due to the difficulty of accurately characterising the concentration and composition of bioaerosols and assessing their ability to spoil and alter the functionality of food. Bioaerosols are further ubiquitous in the environment and vary in concentration seasonally, geographically and by diurnal cycle (Ren *et al.*, 1999), making the interpretation of concentration data problematic and regional recommendations for control based on data even more difficult.

Bioaerosols can be solid, liquid or suspended in liquid droplets and can contain bacteria, bacterial spores, fungi or fungal spores, antigens, toxins, viruses, plant pollens, and faecal matter (Lutgring *et al.*, 1997). In densely populated and enclosed buildings such as schools, microorganisms originating from several strata, if not controlled, accumulate and are easily aerosolised (Donham *et al.*,

1986; American Conference of Governmental Industrial Hygienists, 1989; Duchaine *et a*l., 2000). Organisms regularly associated with bioaerosols have been shown to reach levels of 10^5 to 10^7 cfu.m^{-3} (Clark *et a*l., 1983; Donham *et al*., 1986; Cormier *et al*., 1990; Crook *et al*., 1991). Sources of bioaerosols include waste treatment systems, building maintenance, and bacterial or fungal growth in the environment. In schools, the children may also be a source of bioaerosol contaminants carried on their clothes or skin (Figure 1.1). Faecal contaminants of human origin may also be introduced into schools as a result of poor personal hygiene practices and surrounding farming practices in rural area (Lutgring *et al*., 1997).

Another major impacting factor on the aerosolising and distribution of airborne microorganisms is ventilation systems that directly affect other factors such as relative humidity and temperature, further influencing the viability of bioaerosols present, the time spent airborne, and the size of carrier droplets - all of which determine the bioaerosol composition and contamination levels of exposed surfaces (Heldman, 1974; Meklin *et al*., 2003).

Figure 1.1 Crowded classroom in a primary school

Apart from the possible secondary effects of the airborne microorganisms that contaminate surfaces and food, the three ways in which children in schools are exposed to airborne microbes are through inhalation, direct contact and ingestion (Rogers, 2003).

1.3.1. Inhalation

For an individual to be exposed to microorganisms through inhalation, fungal or bacterial spores must be present in the breathing space. For some types of fungi, spore release into the breathable fraction of air is relatively easy and the slightest movement or vibration can release large amounts of spores. Spores and fungal components could further spread passively in the indoor environment, as they do outdoors, following the flow of air (Rogers, 2003). Likewise bacteria and their

related spores follow similar distribution patterns into the breathable air fraction. However, the number of these organisms in the air are more dependant on humidity and the presence of dust particles (Hyvärinen, 2002).

1.3.2. Contact

Exposure to bacteria and fungal spores can furthermore occur through direct contact. Microbial aerosolisation still occurs as described in the previous section, with the only difference being that the aerosolised pathogens infect the body surfaces such as the skin rather than respiratory tract. *Stachybotrys* spp., for example, could cause dermatitis in humans (Environmental Protection Agency (EPA), 2001).

1.3.3. Ingestion

Once microorganisms are aerosolised they could contaminate food to affect the health of both the food handlers and consumers (Lutgring *et al*., 1997). Airborne organisms that are usually associated with food spoilage and subsequent poisoning include, amongst others members of the *Staphylococcus* genus (Wieser and Busse, 2000).

1.4. Fortified biscuit storage

The fundamental aims of the primary school nutrition programme (PSNP) were to improve education by increasing active learning capacity, school attendance and punctuality by providing an early morning snack; to improve health through micro-nutrient supplementation, parasite control/eradication and through providing

education on health and nutrition; and to enhance broader development initiatives, especially in the area of combating poverty (McCoy *et al.*, 1997).

At many schools in the Free State Province, South Africa, fortified biscuits (Figure 1.2) are served to children as an early morning snack by volunteer workers and are intended to provide 50% of the recommended dietary allowance (RDA) of iron (5 mg ferrous fumarate), iodine (60 μg potassium iodate), and β-carotene (2.1 mg) for children aged 7-10 years (Van Stuijvenberg *et al.*, 2001). A previous study conducted by Van Stuijvenberg *et al.* (1999) demonstrated that consumption of the fortified biscuits led to a significant improvement in micronutrient status and also appeared to have a favourable effect on the morbidity and cognitive functions of the school children.

Figure 1.2 Fortified biscuits served to children in the primary school nutrition programme (PSNP)

These foods are distributed on a monthly basis to both urban and rural schools and are stored in designated areas until consumed. Storage facilities usually entail a corner in a classroom or a chemical or stationery store or cupboard (Figures 1.3 and 1.4). As mentioned, the bioaerosols that prevail in schools have the ability to contaminate not only surfaces but also the stored food products especially when these have been left open (Figure 1.2) (Madl and Morawska, 2003).

Figure 1.3 Overcrowded storeroom utilised for the storage of fortified biscuits

Figure 1.4 Fortified biscuit box stored open in a storeroom of a typical primary school partaking in the primary school nutrition programme (PSNP)

Dust and bioaerosols enter classrooms through various openings such as air intakes, windows (Figure 1.5), doors and cracks in walls (Figure 1.6) (Hyvärinen, 2002; Rogers, 2003). Airborne bacteria and viable fungal spores may subsequently dwell in this environment for various lengths of time, depending on their settling velocity before being deposited on various surfaces that include food (Rogers, 2003). Genera that are frequently isolated from African dust are listed in Table 1.1 (Griffin *et al.*, 2001). These organisms survive in dry conditions and once they have settled on surfaces that contain sufficient moisture and nutrients, they proliferate, just to be aerosolised and distributed once more.

Figure 1.5 Broken window in a primary school taking part in the primary school feeding programme

Figure 1.6 Cracked and moisture-damaged wall in a storage room of a primary school partaking in a feeding programme

Table 1.1 Bacteria and fungi previously isolated from African dust (Griffin *et al.*, 2001)

Genus/species	Type
Arthrobacter globiformis	Bacteria
Bacillus megaterium	Bacteria
Curtobacterium citreum	Bacteria
Sphingomonas sp.	Bacteria
Cladosporium cladosporiodes	Fungi
Coccodinium bartschii	Fungi
Gibberella pulicaris	Fungi
Pleospora rudis	Fungi
Curtobacterium albidum	Bacteria
Curtobacterium luteum	Bacteria
Microbacterium sp.	Bacteria
Pseudomonas riboflavina	Bacteria
Sinorhizobium sp	Bacteria
Sphingomonas trueperi	Bacteria
Bacillus punilus	Bacteria
Kocuria erythromyxa	Bacteria
Microbacterium testceum	Bacteria
Pseudomonas alcalophila	Bacteria
Pseudomonas aleovorans	Bacteria
Sphingomonas pruni	Bacteria
Cochiobolus sativus	Fungi

1.5. Rationale

As the concentration of dust is proportional to seasonal change and droughts one might expect the prevalence of associated microorganisms to follow the same pattern. Likewise the distribution and makeup of these aerosolised microbial populations are influenced by environmental conditions that include humidity (Venter *et al*., 2004). In the Free State Province, South Africa, as is the case elsewhere, dust and its associated microbiota get blown into almost any unsealed environment including the food storage facilities of the PSNP. As biscuits fortified with vitamins, proteins and sugar are stored under sub-optimal conditions prior to consumption in this feeding programme, the mentioned fortified foods will inevitably be contaminated with dust and associated microbiota. Seasonal-related changes in moisture and the occurrence of rain storms further challenge the ability of the poorly-stored hygroscopic fortified biscuits to withstand moisture absorption and subsequently microbial proliferation. Growth of the contaminant bacteria and fungi will in turn not only alter the safety of the products but also the functionality. Therefore, when presenting this food to target consumers who are already malnourished and in many cases immuno-compromised, both acute and chronic impacts on their health may be expected.

1.5.1. Aims and objectives

The aims of the study were therefore:

- to determine the level and distribution of viable airborne microorganisms in the storage rooms and the outdoor environment at both rural (higher dust exposure) and urban schools participating in the PSNP;
- to identify the microbial hazards associated with fortified biscuits and to assess the influence of season on the bioaerosol profile;
- to evaluate the fluctuation in saccharide content of the fortified biscuits served to children;
- to quantify sugar depletion, as a marker for microbial growth on this functional food; and
- in addition, the influence of the geographical localisation of schools on the presence of foodborne pathogens will be assessed.

Data generated in this study will be applied to make recommendations to the Department of Health and fortified biscuits manufactures regarding the quality, safety as well as the storage conditions in the primary schools participating in feeding programmes. This information will further cast light on the ability of common foodborne pathogens to degrade nutrients and proliferate in fortified foods.

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Chapter 2

The influence of season-associated extrinsic factors, geographical localisation and facility design on the distribution of typical airborne indicator organisms in schools participating in a feeding programme

2.1. Abstract

As is the case in the commercial food production environment, the safety and shelf life of food served in school feeding programmes in Africa depends on the initial quality of the product, the storage conditions and the associated preparation practices. The extrinsic factors that dictate the dispersal and survival of foodborne pathogens include temperature, relative humidity and a gaseous atmosphere that prevail not only in the food but also in the aerosol (breathable air) environment. Therefore this paper will pay particular attention to the presence and distribution of indicator organisms in the food storage environment of schools participating in the mentioned feeding programme. Samples of breathable air were collected at fifty schools participating in a primary school feeding programme. At each school eight samples were collected, four (two in the food storage area and two in the environment from which air is fed to the storeroom) during the southern hemisphere winter (dry months, May-September) and four during the summer (wet months, November-April). Besides the preservation of the organoleptic property and the integrity of the food, the objective of proper food storage is to protect food from pests and other contaminating elements such as microbes. To verify and address the concerns raised in this paper it is finally

recommended that the ability of microorganisms to proliferate on the foods (with different water activities) provided to children in feeding programmes be assessed and the menus adjusted in accordance with season-associated changes in the extrinsic factors that could influence microbial viability and growth.

Keywords: Microbiota, storerooms, temperature, relative humidity.

2.2. Introduction

As is the case in the commercial food production environment, the safety and shelf life of food served in school feeding programmes depend on the initial quality of the product, the storage conditions and the associated preparation practices. As many of these programmes have been implemented for more than a decade the microbial populations related to this environment have evolved in a manner concomitant with those in the formal food industry (Venter *et al.,* 2003). In the latter environment the major characteristics that underline the survival of the foodborne pathogens include amongst other things their tolerance of adverse conditions and their relatively small size and related ease of dispersal.

The extrinsic factors that dictate the dispersal and survival of foodborne pathogens include temperature, relative humidity and a gaseous atmosphere that prevail not only in the food but also in the aerosol (breathable air) environment. A strong association between the presence of airborne microbiota and the mentioned extrinsic factors has further raised concern regarding the ability of ventilation systems to distribute and control microbial contaminants in the food production environment (Burge *et al.*, 2002; Chung-Min and Wen-Chang, 2005).

Similarly, facilities that were not initially designed for food storage and preparation i.e. schools, and classrooms in particular, rely on natural moving air through open windows and doors for ventilation. Griffin *et al.* (2001) cautioned however that there are diseases associated with this moving air in Africa that are of particular concern due to the age and susceptibility of the occupants of schools

(Smith *et al.*, 1996; Lee and Chang, 1999; Bayer *et al.*, 2002; Meklin *et al.*, 2002). Though no relation between these diseases and the consumption of food exposed to the moving air are documented, the diseases are brought on by airborne dust particles and aerosolised microbiota (Shelton *et al.*, 2002).

In South Africa, as in many other countries, the general lack of adequate storage facilities and a food distribution infrastructure in school feeding programmes leads to the development of customised functional foods as vehicles for both vitamins and minerals. For similar reasons these functional foods were not only developed with nutrition in mind but were also developed to be "microorganism unfriendly". In the South African primary school feeding programme the latter was achieved by producing biscuits and soup powder for instance both of which have a water activity that is too low to sustain microbial growth.

As the quantity of food required is considerable, these foods are produced in formal commercial food production plants followed by distribution and repackaging by informal sub-suppliers. The repackaging is done by manual labour that forms part of a job-creation initiative and food supplies sufficient for one month are distributed to schools in both urban and rural areas. The schools that participate in the feeding programme then receive a consignment at least once a month and store the food either in empty classrooms (usually in larger urban schools) or in the corners of occupied rooms (usually in smaller rural schools) from where it is distributed to the children. The food is packaged in large quantities in cardboard boxes to minimise the time spent on unloading at schools.

This, however, results in the packaging being opened upon requirement and left open for several days until all has been consumed. During this period the food is exposed not only to the contaminating microbiota associated with the moving air but also to rodents, microbiota from the school occupants and residents in the building, and all the extrinsic factors required for microbial proliferation.

Despite the development of “microbial unfriendly” functional foods for feeding programmes (Van Stuijvenberg *et al*., 2000), the influence of season-associated extrinsic factors, facility design and the geographical localisation of schools on the control and distribution of airborne organisms is not clear. A thorough understanding of microbial survival and distribution in school feeding programmes will thus offer the best opportunity to control the resident microbial populations. This paper will therefore pay particular attention to the presence and distribution of indicator organisms in the food storage environment of schools participating in the mentioned feeding programme. The results will further highlight the importance of adequate food safety management in the industry that supplies food to feeding programmes and will propose practical alternatives to current storage and distribution practices. Finally this paper will comment on the influence of the extrinsic environment at, and the geographical localisation of, schools on the quality of the breathable air and the ability of the associated microbiota to survive, proliferate and contaminate the food stored for feeding children.

2.3. Materials and methods

2.3.1. Sample selection

Samples of breathable air were collected at fifty schools participating in a primary school feeding programme in the Motheo district, Free State Province, South Africa. At each school eight samples were collected, four (two in the food storage area and two in the environment from which air is fed to the storeroom) during the southern hemisphere winter (dry months, May-September) and four during the summer (wet months, November-April). The fifty schools were randomly selected from a 20 000 km^2 geographical area that consists mainly of formal and informal settlements as well as farmland and light industry. Seventy-two percent of the selected schools were situated in rural areas, as the number of schools that take part in feeding programmes in these areas is higher, and 28% of the schools were situated in urban areas.

2.3.2. Air sampling procedure

All microbial samples were collected 1.5m above the floor on 65 mm RODAC plates by means of impaction on soft agar. The (SAS) Super-90 surface air sampler (PBI International, Milan, Italy) was applied for this purpose. The air sampler was calibrated at an airflow rate of $0.03m^3.min^{-1}$ and all detachable parts sterilised before use and between sampling with 70% ethanol (Venter *et al.*, 2004). For isolation of the indicator organisms *Escherichia coli* and *Staphylococcus aureus,* total viable aerobic organisms, and total viable fungi, Plate Count Agar (PCA), Chromocult Coliform Agar (CCA), Baird Parker Agar (BPA) and Potato Dextrose Agar (PDA; Merck, SA; pH=3.5, tartaric acid) were

used respectively. Subsequent incubation of the plates was done at temperatures that ranged from 25 to 37℃ for periods that ranged from 24 to 72h. All colonies were enumerated using the positive-hole correction method and expressed as colony forming units per cubic meter of air sampled.

2.3.3. Facility design

Assessment of the storeroom design and maintenance was done by means of a structured checklist (Appendix B) that also allowed for the notation of observations regarding the storage and related hygiene practices.

2.3.4. Quantification of extrinsic factors

The extrinsic factors that could influence the survival of microorganisms i.e. temperature (Area Heat Stress Monitor-Questemp SA) and relative humidity (Whirling psychrometer, Airflow Instrumentation, SA) were also measured, in addition to factors that could influence the distribution of the assessed microbiota. These factors included air flow (Airflow anemometer - LCA 6000 VT, Airflow Instrumentation, SA) and airborne particle (dust) concentrations (handheld aerosol monitor - 1005/1060, PPM Enterprises, Inc) (Venter *et al.*, 2004). Positive and negative controls were included and all analyses and assays were repeated at least in duplicate.

2.4. Results and Discussion

2.4.1. Summary of the facility design

The results obtained from the technical investigations are presented in Table 2.1. From these results, it is clear that the storerooms used for the storage of food in the mentioned feeding programme are not designed and maintained according to good storage practices. The majority of the investigated storerooms were also not designed to provide the required barriers against moisture, temperature, pests, dust and the associated microbes. Therefore limited control over the quality and safety of the food stored under the noted conditions would be expected.

2.4.2. Season-associated extrinsic factors

The environmental factors that influence the viability and distribution of microorganisms present in the storerooms are listed in Figure 2.1. From these results it is evident that the storerooms (on average) provide an environment that would sustain microbial viability concomitant to proliferation, given that a suitable substrate and sufficient time is available. Though products such as biscuits and soup powder, which would limit microbial growth due to a low water activity, are stored in the mentioned storerooms, other food products stored include bread, peanut butter, and fruit juice: all of which could sustain microbial growth if not properly preserved (McCoy *et al*., 1997; Van Stuijvenberg *et al*., 2000; Venter *et al*., 2003).

Table 2.1 The storage facility design and practices in schools (rural and urban) participating in a feeding programme in South Africa

	Occurrence in Rural (%) n=36	Occurrence in Urban (%) n=14
Storage facility design		
Ceiling	64	100
Air bricks with filters	3	29
Ventilation through natural moving air	92	100
Ventilation through damaged windows	11	50
Storage size (more than 4.4m^2)	89	36
Facility cleanliness		
Organised storerooms	75	36
Clean storerooms	44	71
Foodstuffs storage only	0	7
Storage practices		
Food stored on shelves	39	64
Food stored in original opened packaging	100	100

Food with low water activity tends to be hygroscopic and if exposed for prolonged periods to relative humidity levels above 55% (Figure 2.1) would absorb sufficient amounts of moisture to sustain microbial growth (Pasanen *et al.*, 2000; Baylis *et al.*, 2004; Schrödter, 2004). In the evaluated storerooms these levels of relative humidity were however usually reached during the summer months and only occasionally during winter. This corresponds to the rainfall patterns of this region.

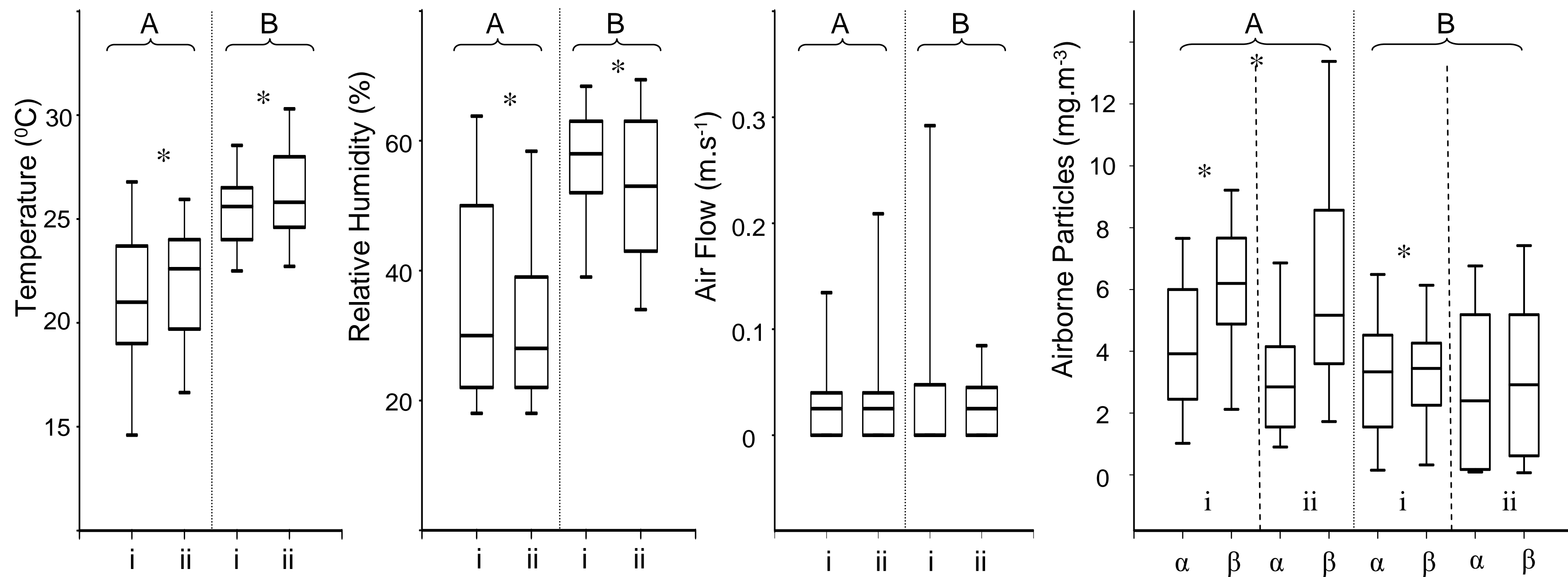

Figure 2.1 Environmental factors quantified during the dry (A) and wet (B) seasons within (α) and outside (β) storage facilities of primary schools participating in a feeding programme situated in both a rural (i, n=35) and an urban (ii, n=15) environment

* A significant difference (P≤0.05) between adjacent parameters

With this information available, the ability of these storerooms to control relative humidity (RH) was assessed by quantifying the change in the average RH over the seasons for both rural and urban schools combined. From this it was evident that the storerooms had limited control over the season-associated change in relative humidity ($\Delta \bar{x}$ relative humidity = 27.69 %).

For both seasons assessed the average temperatures inside the storerooms varied from 22 to 26ºC which is conducive to mesophilic organism growth. The small temperature fluctuation ($\Delta \bar{x}$ temperature = 4ºC) nonetheless signifies the ability of the majority of these storerooms to control temperature as outside day temperatures in this part of the continent could easily fluctuates between 12 and 35ºC. However, these storerooms, though poorly designed and maintained, controlled to a large extent the season-associated change in air flow ($\Delta \bar{x}$ air-flow = 0.01m.s^{-1}) and to a lesser extent the season–associated change in airborne particle concentration ($\Delta \bar{x}$ [airborne-particle] = 1.23 mg.m^{-3}).

2.4.3. Geographical localisation and airborne indicator organism presence

Microorganisms indicative of poor food manufacturing practices are defined as indicator organisms for the purpose of this paper. In the formal food industry, *Escherichia coli* indicates the presence of faecal contamination; *Staphylococcus aureus,* extensive human handling; and total viable aerobic organisms, poor process hygiene (Department of Health, 2000). Whether the presence of these organisms in the breathable air of the mentioned storerooms at schools would

be indicative of similar practices is questionable as none or little food processing (depending on the product served) actually occurs at the schools or in the storerooms. In this scenario the presence of *E. coli* would be associated with faecal matter presence related to animals and rodents in the vicinity of the schools - especially in the rural areas. The presence of *S. aureus* in the breathable air could result from the same sources as mentioned for *E. coli* and could also be associated with aerosolised droplets and sprays originating directly from the occupants of the school. As the majority of fungi are able to survive in environments with a low water activity, their presence and the concentration of the total viable aerobic organisms were assessed to cast light on the ability of these storerooms to sustain microorganisms. This data will also provide information to Environmental Health Practitioners on the allerginicity and possible presence of endo- and mycotoxins in the breathable air of these schools.

As noted in Figure 2.1, airborne dust though at various levels was present in all storerooms during all seasons. It was therefore assumed that if the airflow in- and outside the storerooms were sufficient to carry dust particles the same would be true for viable bacteria, fungi and fungal spores (Figure 2.1). The results presented in Figure 2.2 supported this assumption but could not be presented as a factor of the airflow in this study. With the exception of *E. coli* (only occasionally isolated during the dry season and absent during the wet season) the average bacterial and fungal counts varied in general between 10^1 to 10^2 $CFU.m^{-3}$. Compared to the available literature, these counts were fairly low and apart from a meagre allergen threat, would pose a limited threat as source for endo- and

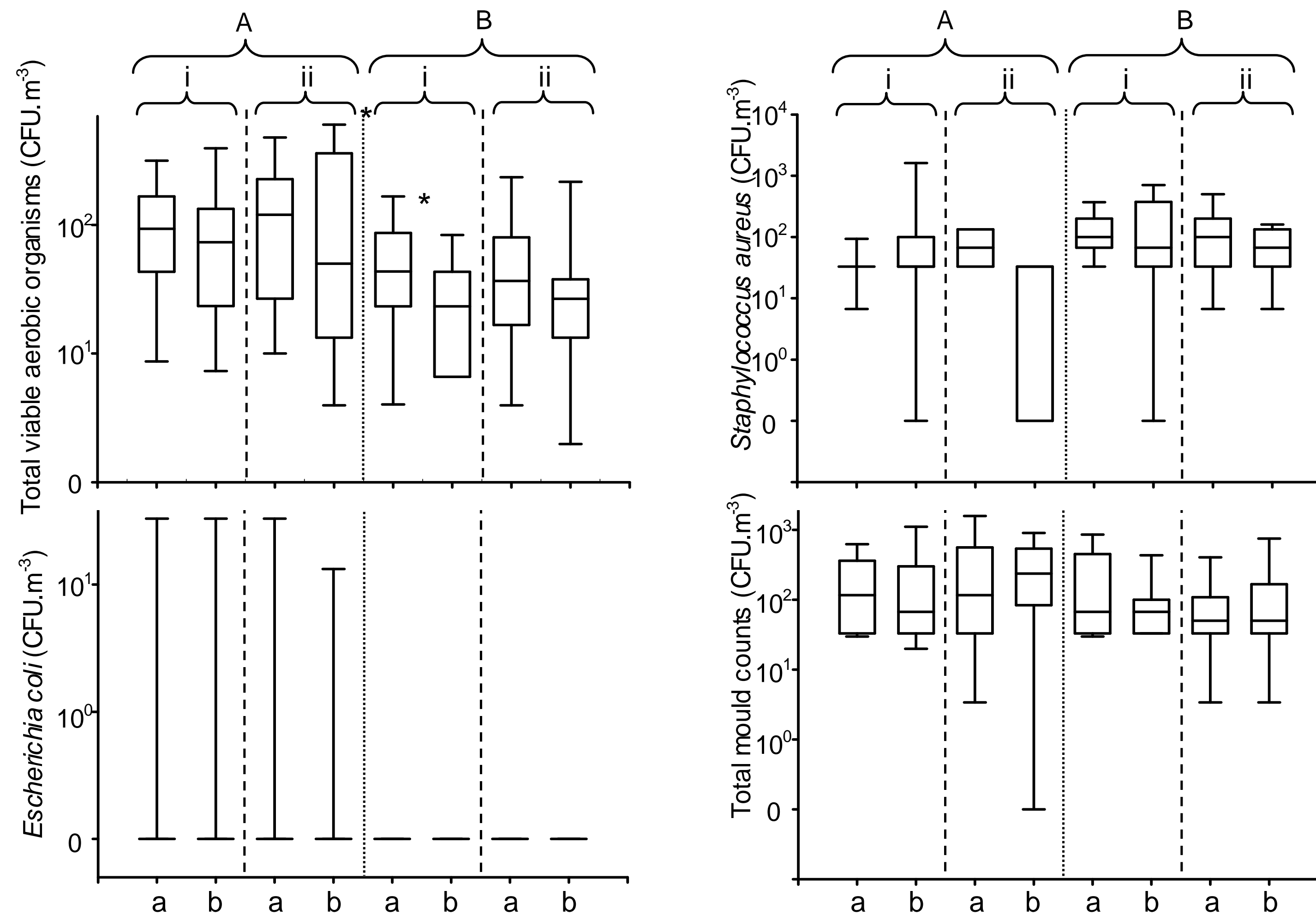

Figure 2.2 The average culturable airborne microorganisms isolated from breathable air in primary schools (located in rural (i) and urban (ii) areas) participating in a feeding programme in Central South Africa during both the dry (A) and wet seasons (B) in storage rooms (a) and the outdoor environment (b)

* A significant difference ($P \leq 0.05$) between adjacent parameters

mycotoxin whilst aerosolised (Shelton *et al.*, 2002; Toivola *et al.*, 2002).

Other than in the case of rural storerooms assayed during the wet season no significant impact of storerooms in the mentioned feeding programme on airborne bacterial and fungal profiles was noted (Fig. 2.2). Likewise, with the exception of total viable aerobic organism counts, seasonal change had no significant influence on the airborne organism profiles. From this it would seem that the schools in question boast a resident bioaerosol population that is not significantly influenced by the noted environmental parameters. Though the source of the bioaerosols assayed is not clear, the low levels of *E. coli* nonetheless indicate a limited presence of aerosolised faecal matter. In addition, *S. aureus* probably results from aerosols dispersed by the school occupants. From the latter information and the presence of fungi and total viable counts in the breathable air fraction, it could also be noted that though the microbial counts were low, the storerooms provided an environment conducive to microbial survival as aerosolised particles and subsequently as food contaminants.

Finally, the influence of geographical localisation (urban/rural) and storeroom design on internal temperature, relative humidity and dust particle concentrations was significant ($P \leq 0.05$). In general, storerooms in rural schools had a lower temperature and increased relative humidity and airborne particle counts. On the other hand, locality had a limited influence on the organisms present in and outside the storerooms. This implies that a change in locality and hence environment, does not necessarily imply a change in aerosolised microbiota but

rather that the organisms isolated are more closely related to internal activities and the presence of the occupants.

2.5. Conclusion

Besides the preservation of the organoleptic property and the integrity of the food, the objective of proper food storage is to protect food from pests and other contaminating elements such as microbiota. In an environment where good storage practice is not possible due to a lack in infrastructure, special care should be taken regarding the kind of food stored and packaging material used, to ensure food safety. Environments typifying this kind of situation are to be found in schools participating in feeding programmes. From our results it may be concluded that though the storage facilities at the schools in question are, in terms of all technical data, not suitable for food storage, they did seem to govern some of the extrinsic factors that influence microbial viability. No control of these storerooms regarding the resident airborne bacteria was however noted. Specific attention should be given to the development of functional packaging that could, for example, be re-sealed after being opened, as was given to the development of functional "microbial unfriendly" foods. The results presented in this paper further identified the occupants of the schools as the possible source of the organisms present in the storerooms and not the building or the surrounding environment. This would also call for food storage in facilities that are separated or isolated from the occupants, or for more suitable packaging. Manufacturers of the food for feeding programmes are further cautioned not to rely solely on the properties of food, such as a reduced water activity, as safeguard, as the inability

of the assessed storerooms to control humidity fluctuations could result in the food being unsuspectingly hydrated. It was further noted that in South Africa unlike other more humid countries, the facility design and geographical localisation had a limited influence on the resident bioaerosol profiles.

To verify and address the concerns raised in this paper it is finally recommended that the ability of microorganisms to proliferate on the foods (with different water activities) provided to children in feeding programmes be assessed and the menus adjusted in accordance to season-associated changes in the extrinsic factors that would influence microbial viability and growth. In this environment, aerosolised endo- and mycotoxins are also expected to be low and thus pose a limited threat. The same might not be true for allergies as continuous exposure of the occupants to these levels of fungi and fungal spores could lead to gradual sensitisation to these allergens. It should further be noted that the occupants of the mentioned schools are probably not knowledgeable regarding proper food storage practice and are probably unaware of the threats posed by the resident microbiota. This problem could be dealt with by providing proper storage instructions printed on the boxes or included as an insert in the container.

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Chapter 3

Sucrose Breakdown Associated with Staphylococcal Growth on Fortified Biscuits

3.1. Abstract

Among the functional foods available to the world population today are fortified biscuits supplied to school children in feeding programmes in developing countries. A lack of adequate storage and transport infrastructure for fortified biscuits every so often results in an increase in moisture and a subsequent increased water activity. Under such conditions the saccharide-rich biscuits provide an ideal substrate for sustaining bacterial proliferation. Therefore the aim of this study was to quantify the species of *Staphylococcus* present on fortified biscuits served to children in a feeding programme in Africa and to establish their saccharide degradation patterns. Biscuit samples were collected from primary schools participating in a feeding programme in Central South Africa during the dry and the wet seasons. These biscuits were analysed for total viable counts, *Staphylococcus* sp., *Escherichia coli*, and aerobic and anaerobic plate counts. High performance liquid chromatography (HPLC) was further applied to quantify sugar degradation in fortified biscuits by the isolated organisms. The first part of this study showed that the fortified biscuits served to children at primary schools in South Africa tend to be contaminated irrespective of the season of manufacture. The types of contaminant further prove that handling by humans, probably during packaging, is the major source of contamination.

From the results it was further concluded that the functionality of fortified biscuits decreases severely in the presence of staphylococci over the first three days of growth. This does not coincide with an increase in bacterial numbers and does influence the safety of the product (based on staphylococcal counts). The bacteria do however adapt and increase their growth rate after a further twelve days. If thus consumed after a 21-day period, biscuits exposed to moisture would be unsafe.

Keywords: Schools, HPLC, fortified biscuits, *Staphylococcus* sp.

3.2. Introduction

Among the many functional foods available to the world population today are fortified foods supplied to children in feeding programmes in developing countries. In terms of the foods supplied to children the fortification vehicle of choice is often reported to be fortified biscuits. These biscuits are intended to provide 50% of the recommended dietary allowance (RDA) of several micronutrients, saccharides and protein (Van Stuijvenberg *et al.*, 2001). Biscuits are further recognised as a safer product with a prolonged shelf life as they have a low water activity that does not sustain bacterial growth (Van Stuijvenberg and Benadé, 2000).

A lack of adequate storage and transport infrastructure for fortified biscuits every so often results in an increase in moisture and subsequent increased water activity (Child Health Unit (CHU), 1997). Under such conditions the saccharide-rich biscuits provide an ideal substrate for bacterial proliferation. The utilisation of saccharides as a carbon source by heterotrophic contaminating bacteria is well described (Schlegel, 1993). Many bacteria are able to catabolise simpler saccharides such as glucose and some bacteria may even utilise more complex polysaccharides such as lactose and starch (Madigan *et al.*, 1997). The oxidation and fermentation of saccharides usually result in the production of organic acids as well as the required energy to sustain bacterial proliferation and survival (Tortora *et al.*, 1995). Furthermore, various bacterial groups have evolved to transport complex saccharides from their surrounding environment across the membrane into the cell and has acquired the ability to degrade these molecules.

The ability of these different bacterial groups to produce metabolic by-products from saccharide catabolism is also diverse (Schlegel, 1993).

The bacterial genus investigated in this study is the ubiquitous *Staphylococcus*, frequently present in the environment where saccharide-rich fortified biscuits are stored (Chapter 1). It has been reported that this genus, while proliferating on food, usually does not result in undesirable organoleptic properties, yet is able to produce several enterotoxins (Concon, 1988). Most etiological surveys have assigned the source of many staphylococcal species to humans and animals (Pierson and Stern, 1986). *Staphylococcus aureus* is considered to be highly osmotolerant as it is able to grow at a water activity level as low as 0.86 (Gould, 1995).

The presence of starch and protein in considerable amounts is able to enhance toxin production by staphylococci (Frazier and Westhoff, 1988). In a functional food product such as the mentioned fortified biscuits, proliferation of this group of organisms will not only lead to a loss in functionality but will also render the product unsafe for human consumption. Therefore the aim of this study was to quantify the species of *Staphylococcus* present on fortified biscuits served to children in a feeding programme in South Africa and to establish their saccharide degradation patterns. This information would prove valuable in the future development of fortified biscuits and in understanding the process of saccharide catabolism by different staphylococcal species. In addition, three indicator organism groups that included total viable counts, *E. coli* and aerobic and

anaerobic spore formers, were isolated from the biscuits in order to determine the origin of the contaminants.

3.3. Material and methods

3.3.1. Sampling site and procedure

Randomly selected samples of 100 fortified biscuits (Appendix 1, Figure 5.4) weighing 23g each were collected from fifty schools participating in the primary school nutrition programme in the Motheo district, Free State Province, South Africa. Samples were aseptically collected and transported to the laboratory on ice. The samples were collected during the dry (winter: May-September) and the wet season (summer: November-April).

3.3.2. Sample preparation and bacterial analysis

From each biscuit collected, 10g were blended in sterile saline solution (90ml) and homogenised using a stomacher (Seward Stomacher 400). Further dilutions were prepared in saline solution and 0.1ml aliquots were plated on the media described below using the spread-plate method (Herbert, 1990; Martínez-Tomé *et al*., 2000). All analyses were done at least in duplicate.

Total Viable Counts

Total Viable Counts were enumerated by using Plate Count Agar (PCA, Merck-SA) and the plates were incubated at 25°C for 48 hours (Bryan *et al*., 1996).

Escherichia coli

For the enumeration of *E. coli* Chromocult Coliform Agar was used and the plates were incubated at 35°C for 48 hours (Martínez-Tomé *et al.*, 2000).

Staphylococcus sp.

For the isolation of *Staphylococcus aureus,* Baird-Parker Agar (Biolab-SA) was used and plates were incubated at 35°C for 48 hours. Further identification of *Staphylococcus* sp. was done by transferring colonies from the Baird-Parker Agar (non-*S. aureus*) to Blood Agar followed by incubation for 24 hours at 35°C. These isolates were subsequently identified using the API-Staph system (Bio Mérieux, France; Nagase *et al.*, 2002) and APILAB software in accordance with the manufacturer's instructions.

Aerobic and Anaerobic Plate Counts

Spore-forming bacteria were isolated by transferring colonies isolated on PCA to a saline solution and exposing them to a temperature of 80°C for 10 minutes to destroy the vegetative cells. Thereafter they were incubated on Plate Count Agar plates (Harrigan, 1998). Anaerobic populations were quantified by incubating in anaerobic jars at 25°C for 24 hours (Anaerobic A Merck; Martínez-Tomé *et al.*, 2000).

3.3.3. Sugar depletion by isolated *Staphylococcus* sp.

Five *Staphylococcus* species (*S. aureus, S. cohnii*, *S. homonis*, *S. lugdunensis* and *S. xylosus*) were isolated from the fortified biscuits. These organisms were

transferred into nutrient broth, incubated at 35°C for 24 hours and inoculated into flasks containing 300g ground biscuits suspended in 500ml sterilised water. Each flask was incubated at 25°C for two weeks. Sugar analysis was conducted seven times for each flask at three-day intervals.

3.4. Sugar analysis

3.4.1. Reagents and standards

All chemicals used for sugar extractions and preparation were of analytical reagent grade and included HPLC grade ethanol and deionised water (Merck, SA). The sugars standards (sucrose, fructose and glucose) were acquired from Sigma, SA (Chávez-Servín *et al.*, 2004).

3.4.2. Sample preparation

Ten grams of suspended biscuit from the flasks containing *Staphylococcus* sp. were transferred to 100ml ethanol. The contents were vortexed at room temperature for approximately 10 min (until sample was completely dissolved). The solution was filtered through a 0.2 µm nylon filter (Tracer Barcelona, Spain), passed through water plus sugar pak 1 column (360 mm X 7.8 mm diameter) attached to the HPLC system (Chávez-Servín *et al.*, 2004).

3.4.3. Instrumentation and chromatographic conditions

The chromatographic analyses were carried out with a Shimadzu high-performance liquid chromatograph equipped with a refractive index detector. Chromatographic separation was achieved with a mobile phase of deionised

water. The flow-rate of the eluent was 0.5 $ml.min^{-1}$ and the volume of the sample injected was 20µl (filling the loop completely) (Vendrell-Pascuas *et al.*, 2000). Column temperature was maintained at 25℃. Peaks we re identified by comparing retention times with sugar standards. Calibration curves for each sugar was prepared at seven levels, from 0.5 to 10 $mg.ml^{-1}$ for fructose, glucose and sucrose, all dissolved in ethanol-water (1:1, v.v) (Chávez-Servín *et al.*, 2004).

3.5. Results and discussion

The profile of the organisms present on the fortified biscuits served to children at the mentioned primary schools is shown in Figure 3.1. The presence of these organisms shows, without doubt, that these biscuits are contaminated after manufacture (which usually entails high temperature baking that sterilises the biscuits), through processes that involve extensive human handling. From these results it is clear that with the exception of *S. aureus* the microbial load on the biscuits was lower during the wet season than the dry season ($P \leq 0.05$). These biscuits are usually packed by hand and exposed to bioaerosols in the packaging plant and at schools.

Profiling the staphylococcal species on food further emphasised the role of personal hygiene in the manufacturing of food; *S. hominis,* for instance, is frequently isolated from open wounds and cuts (Sancho *et al.*, 1996; Euzeby, 2003).

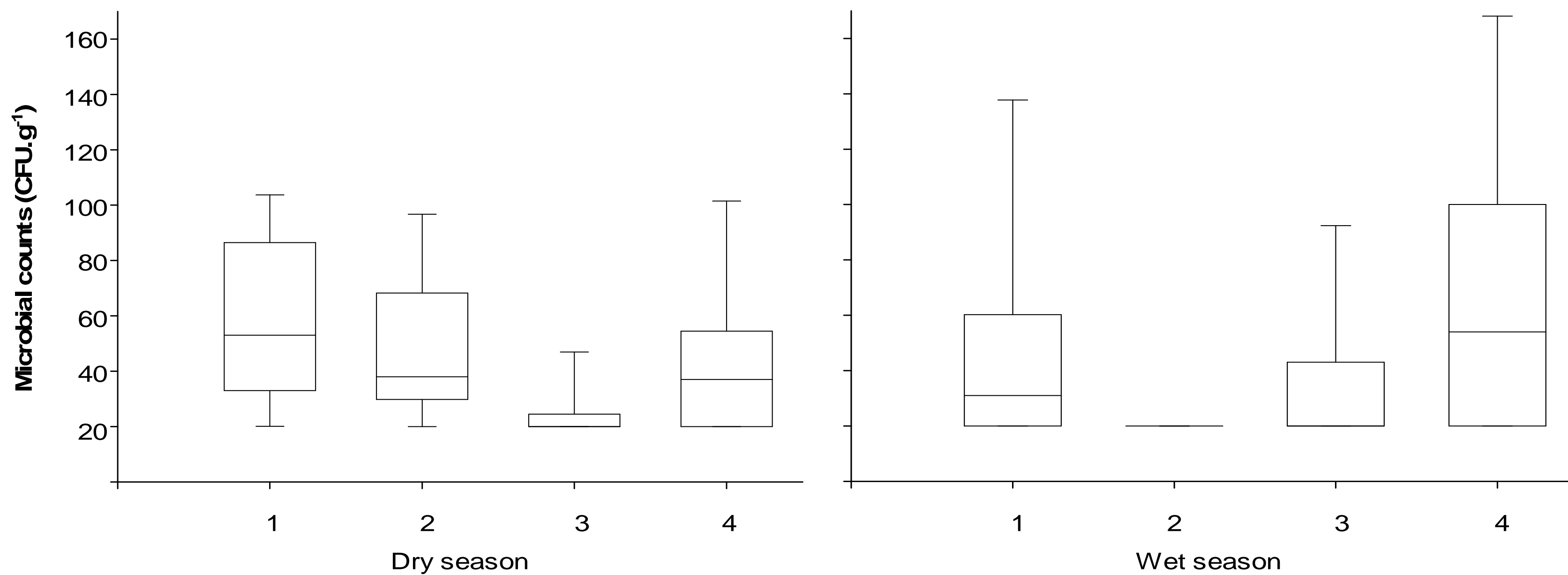

Figure 3.1 Distribution of microorganisms during the dry and wet seasons. (1) Total Viable Counts (TVC), (2) *E. coli*, (3) *Staphylococcus* sp. and (4) Aerobic spore formers

*A linear scale was used to show the standard deviation

Species such as *S. cohnii* and *S. xylosus* have been reported to originate from animals, indicating the possible presence of rodents or other animals in the biscuit-processing and handling environment. All these organisms were present on the biscuits from both seasons.

It is unlikely that these organisms proliferate on the fortified biscuits to produce Staphylococcal endotoxins as the biscuits have a low water activity (Den Aantrekker *et al*., 2003). These biscuits are however stored at primary schools for up to four weeks in conditions that do not prevent spoilage by increased levels of moisture, or in many cases, by rain (Chapter 2). Therefore the mere fact that these biscuits could not sustain microbial life after manufacture (Manley, 1998) should not be seen as the norm for stored biscuits as the threat of microbial growth is not eliminated.

It was hypothesised that high levels of sugars in the presence of sufficient amounts of water would act as a sufficient carbon source for staphylococci in the test flasks containing fortified biscuits (Figure 3.2). As expected, sucrose breakdown occurred rapidly within the first nine days in all flasks but slowed down to undetectable levels over the following twelve days. The viability of the staphylococci decreased concomitant with the saccharide breakdown. After nine days, as the sucrose levels decreased, the growth of all the staphylococci analysed increased slightly, with the exception of *S. xylosus.*

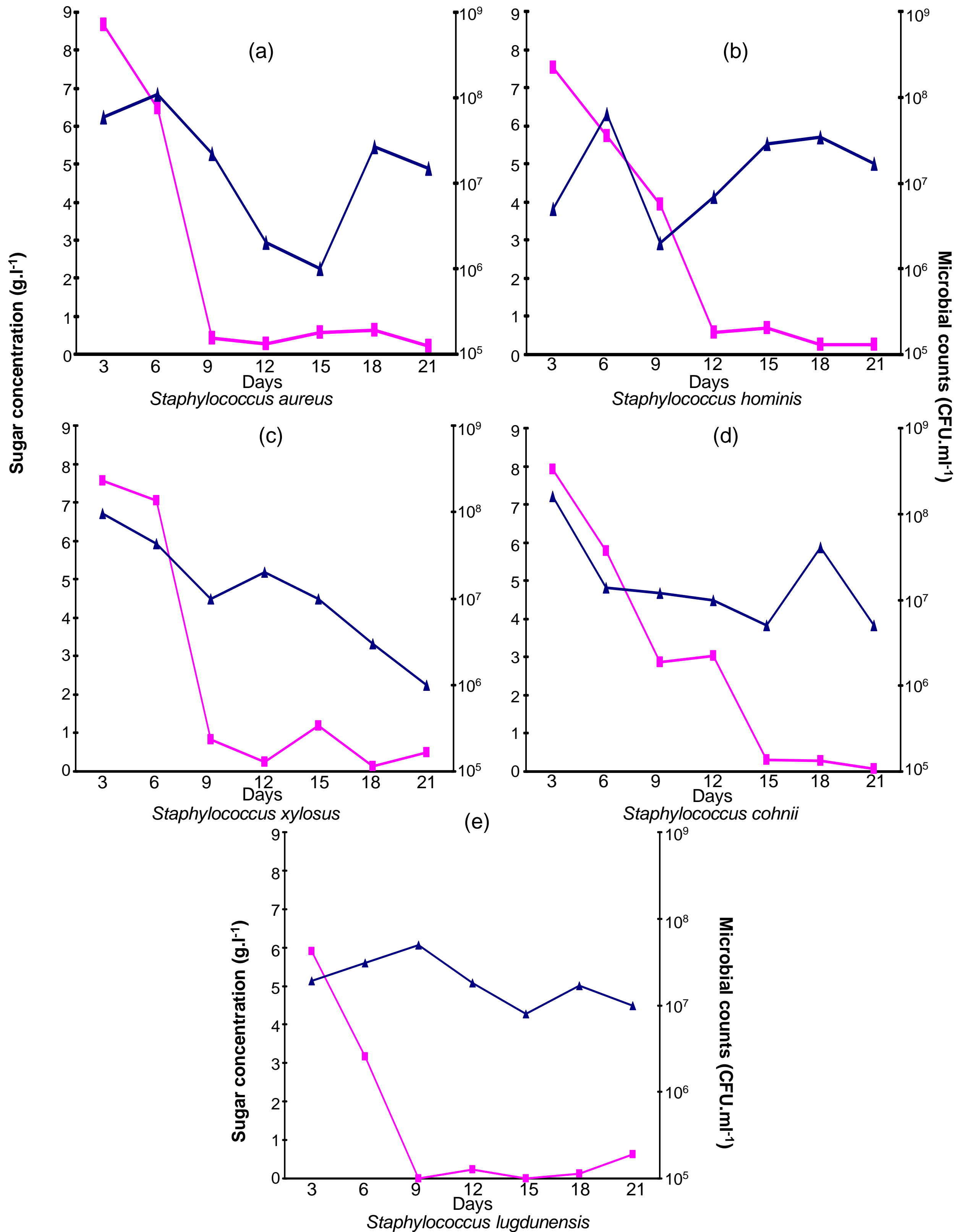

Figure 3.2 The concentration of sucrose(■) together with microorganisms(▲) in fortified biscuits at different time intervals

S. aureus is an osmotolerant bacterium that is known to resist a low water activity resulting from a high salt concentration. The results depicted in Figure 3.2 however support the statement by Vilhelmsson and Miller (2002) that this bacterium is less tolerant to sucrose-induced than salt-induced osmotic stress. In fact, high quantities of sucrose decrease the viability of *S. aureus* in foods. From the results obtained for staphylococcal viability on fortified biscuits it is furthermore clear that the susceptibility of the other species analysed coincides well with that of *S. aureus.*

Enzymes production and breakdown product formation during growth on the biscuits were not investigated but small quantities (1g/l) of fructose and glucose were detectable after 21 days in the flasks of *S. aureus, S. xylosus* and *S. cohnii*. The sucrose breakdown was however prominent over the first three days resulting in a definite change in the functionality of the fortified biscuits. The concomitant decrease in staphylococcal viability further resulted in a product that is probably safer in bacterial quantity terms after the first few days of growth.

3.6. Conclusion

The first part of this study showed that the fortified biscuits served to children at primary schools in South Africa are contaminated irrespective of the season of manufacture. Further, the types of contaminants suggest that handling by humans, probably during packaging, is the major source of contamination. The second part of this study evaluated the ability of the staphylococci species (osmotolerant) isolated from these biscuits to proliferate once the water activity

increased. This simulation was performed as poor storage facilities regularly lead to the biscuits being exposed to increased moisture increase and also directly to rain (leaky roofs).

From the results it was concluded that the nutritional value of fortified biscuits decreases in the presence of staphylococci over the first three days. This does not coincide with an increase in bacterial numbers and does influence the safety of the product (based on staphylococcal counts). The bacteria do however adapt and increase their growth rate after a further twelve days. If thus consumed after a 21-day period, biscuits exposed to moisture would be unsafe. Further investigation is however required to establish the growth of other common bacterial contaminants on the biscuits and their concomitant sugar degradation ability.

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Chapter 4

Characterisation of Airborne Fungi Isolated at Schools Participating in a Feeding Programme in South Africa

4.1. Abstract

Bioaerosols are defined as airborne solid or liquid particles that may contain bacteria, bacterial spores, fungi or fungal spores, antigens, viruses, plant pollutants and faecal material. In schools where food is stored the facilities have not been designed to control bioaerosols through the management of airborne dust, moisture or temperature. Therefore, this study evaluated the influence of facility design and location of schools participating in feeding programmes on the fungal composition, population and quantity within the bioaerosols present. The surface air sampler (SAS) Super-90 with 65 mm RODAC plates containing potato dextrose agar-pH=3.5 (PDA) was applied for fungal isolation from the breathable air. Samples were collected from storerooms at schools participating in a feeding programme in Central South Africa and the outdoor environment during the dry and the wet seasons. The most common fungal genera that were isolated during the dry season were also found in the wet season (i.e. *Penicillium, Alternaria, Cladosporium, Scedosporium*, *Oidiodendron, Trichoderma, Chrysolinia, Phoma, Mucor, Exophiela, Botrytis* and *Oedecephelum*). The presence of these fungal species in the storerooms might be the result of microbes being carried on clothes and subsequently being transferred onto the biscuits. Furthermore, in addition to building construction, crowding in school buildings probably plays a role in affecting microbial levels and fungi found in indoor air. For example, some

of the schools also use classrooms as storerooms because of limited space. The rural schools evidenced a higher number of airborne fungi than urban schools during both dry and wet seasons: this might be due to the greater movement of air. The way rural schools are built may also contribute to the presence of fungi, with the lack of building maintenance observed in some schools possibly also resulting in roof leaks and/or water leaking through the outer walls.

Keywords: Bioaerosols, fungi, school, feeding programme

4.2. Introduction

Bioaerosols are defined as airborne solid or liquid particles that may contain bacteria, bacterial spores, fungi or fungal spores, antigens, viruses, plant pollutants and faecal material (Lutgring *et al*., 1997; Douwes *et al*., 2003; Venter *et al*., 2004). When not controlled, bioaerosols may result in respiratory infections and decreased lung function, as well as in respiratory and cardiovascular mortality in humans (Husman *et al*., 2002; Santilli and Rockwell, 2002; Shelton *et al*., 2002; Toivola *et al*., 2002). Airborne fungi and fungal spores emanate from many sources, which include fungal growth on building surfaces (Miller *et al*., 2000; Shelton *et al*., 2002). Several reports have also identified moving air as a vector for fungi that liberally contaminate indoor surfaces (Rogers, 2003).

In schools participating in feeding programmes in South Africa, such surfaces are usually desks and open and closed cardboard boxes used for food storage (Venter *et al*., 2003). The type of food generally stored in these boxes is biscuits, fortified with micro-nutrients and vitamins, that are stored for up to 30 days. Fungi capable of proliferating on low water activity foods such as fortified biscuits usually include *Penicillium* and *Aspergillus* (Gock *et al*., 2003). Etiological studies conducted in Denmark identified the sources of these genera as wooden substrate, concrete and plaster (Nielsen, 2003). It has also been reported that fungi representing *Penicillium*, xerophilic *Aspergillus* and *Chrysospororium xerophilum* produce toxins while proliferating on food (Gock *et al*., 2003). In 2001 the growth of mycotoxin-producing fungi resulted in several food poisoning cases

in children fed through a feeding programme in the Eastern Cape, South Africa (Medical Research Council (MRC), 2001).

The influence of facility design on the composition of airborne fungal populations is well known (Meklin *et al*., 2002). In food production environments, a strong correlation exists between the efficiency of ventilation systems and the concentration of bioaerosols (Venter *et al*., 2004). Likewise the concentration of airborne dust and moisture, in many cases influenced by temperature, also influences the presence of bioaerosols (Heldman, 1974). In schools, food is stored in facilities that have not been designed to control bioaerosols through management of these parameters. Therefore, the hypothesis for this study is that the facility design and the location of schools participating in feeding programmes could influence the fungal composition, population and quantity within the bioaerosols present.

4.3. Materials and methods

Bioaerosol samples were collected from fifty (36 rural and 14 urban) primary schools participating in a feeding programme in the Motheo district, Free State Province, South Africa. Samples were collected from indoor breathable air in storerooms and the air fed into the storerooms from the outdoor environment. This study was repeated during both dry (winter: May-September) and wet seasons (summer: November-April).

4.3.1. Air sampling procedure

The surface air sampler (SAS) Super-90 was applied to collect all the culturable airborne fungi through impaction on agar (Clark *et al*., 1983; Donham *et al*., 1986; Haglind and Rylander, 1987; Donham *et al*., 1989; Cormier *et al*., 1990; Heederik *et al*., 1991; Thorne *et al*., 1992). All detachable parts were pre-autoclaved and disinfected with 70% ethanol between samples (Venter *et al*., 2004). The air sampler was calibrated at an airflow rate of 30 $l.min^{-1}$. All samples were collected 1.5m above the floor and ground surfaces. Potato Dextrose Agar-pH=3.5 (PDA) in 65 mm RODAC plates (Appendix, Figure 5.2) was used for the collection of airborne fungi, and by incubation for 72 hrs at 28°C followed (Beever and Bollard, 1970).

4.3.2. Fungal identification

All isolated fungal colonies were purified on PDA and transferred to plate count agar. Microscopic examination of these fungi was conducted with a Nikon phase contrast light microscope (Nikon Eclipse E600, IMP). Fungal identification was performed by using the prescribed identification keys by Malloch (1997) which are based on the phenotypic characteristics of the isolated fungi.

4.3.3. Environmental factors

All the environmental factors that generally influence bioaerosol composition were evaluated (Chang *et al*., 2001). Temperature, relative humidity, wind velocity, and dust particle concentrations were measured, concomitant with the bioaerosol sampling. For temperature an area heat stress monitor (Questemp)

was used; for relative humidity a whirling psychrometer (Zeal p2520, Airflow Instrumentation); for wind velocity an airflow anemometer (LCA 6000 VT, Airflow Instrumentation); and airborne particle concentrations were analysed with a hand-held aerosol monitor (1005/1060, PPM Enterprises, Inc) (Venter *et al.*, 2004). All the results reported in this paper are the means of at least four repetitions.

4.4. Results and discussion

In America at least 50% of all children suffering from asthma are allergic to fungi (O'Connor *et al.*, 2004). These fungi include the genera *Alternaria, Aspergillus, Cladosporium* and *Penicillium.* Across all the schools sampled in the current study, the culturable fungi most commonly found in outdoor samples were *Alternaria, Cladosporium,* and *Penicillium* (Table 4.1). The indoor samples had a similar fungal profile, although some genera were more common indoors than outdoors (Table 4.1).

Furthermore, there was a notable season–to–season variation not only in the counts of both indoor and outdoor fungi (Chapter 2), but also in their absence-

Table 4.1 Airborne fungal genera isolated from primary schools participating in a feeding programme in Central South Africa during both dry and wet seasons inside and outside storerooms

	Dry Season						Wet Season					
	Urban Schools			Rural Schools			Urban Schools			Rural Schools		
Fungal Genera Identified	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Penicillium	10	60	40	27	81	19	11	36	64	35	57	43
Alternaria	7	57	43	11	27	73	11	55	45	7	100	-
Cladosporium	1	100	-	6	50	50	2	-	100	6	33	67
Scedosporium	2	100	-	4	50	50	-	-	-	2	50	50
Oidiodendron	3	67	33	8	63	37	1	100	-	1	100	-
Trichoderma	3	67	33	4	100	-	2	50	50	4	25	75
Chrysolinia	1	100	-	1	100	-	-	-	-	-	-	-
Phoma	-	-	-	2	100	-	-	-	-	-	-	-
Mucor	1	100	-	1	100	-	-	-	-	1	100	-
Exophiela	-	-	-	1	100	-						
Epicoccum	-	-	-	1	-	100	-	-	-	-	-	-
Botrytis	-	-	-	1	100	-	-	-	-		-	-
Oedecephelum	-	-	-	1	-	100	-	-	-	-	-	-

A=number of schools where fungus was isolated B=presence in storage rooms (%)

C=presence in the outside environment (%)

presence. The data presented in this paper supports previous reports on the nearly ubiquitous nature of the above mentioned fungal genera. Of these, *Penicillium* and *Cladosporium* were found more commonly in rural than urban schools. Both these organisms can grow on plant stems and leaves, from where they are widely dispersed in the outdoor air.

The presence of fungi in the outdoor environment varied over time in relation to many extrinsic factors that include temperature, relative humidity, airflow and airborne particle concentration, furthermore indoor concentrations may further be influenced by temporal variations in air exchange with the outdoors. Rapid and extensive variations in the mentioned extrinsic factors complicate the quantitative analysis of airborne fungi to such an extent that it has resulted in no universally accepted metric for summarising airborne fungal profiles in a particular locality. Therefore the results from this study should continuously be viewed constantly in relation with the environmental parameters described in Chapter 2.

The categorisation of an individual school as a high-risk (fungi-associated) environment not only for occupants with asthma but also for the food being stored opened, on the basis of a single seasonal sampling collection is further complicated by the relation of indoor fungal levels with those outside at any given time. The indoor fungal level is a direct result of the outdoor fungi penetrating into the schools and storerooms through doors, windows and other sites of air exchange (Chapter 2). As a result, more fungi were concentrated and consequently isolated from inside the class and storerooms than outside.

In areas that are more humid than the localities investigated in the current study, a direct correlation between fungal presence and dampness has been noted (Katial *et al.*, 1997). The exact opposite was however true for the rural schools evaluated in this study, and season-associated humidity fluctuations had a limited influence on the total fungal loads of urban schools. There was however a closer relation with dust, which supports reports that several fungal genera are closely associated with dust and dust events in Africa.

4.5. Conclusion

From the results it is evident that both the occupants of the schools and the food stored open in their original containers are exposed to several fungal genera that could cause allergies and that could proliferate on the food (in the presence of moisture). Moreover, these fungi are more closely related to dust than humidity and probably originate from the outdoor environment which explains the influence of locality of the schools on the fungal population profile. Control of the airborne fungal load and population profile in these schools will be virtually impossible as the only ventilation available for the occupants is natural moving air. Care could however be taken to reseal the food stored (for distribution to the occupants) as this would not only limit the contamination levels of the food but also the exposure of the food to moisture.

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Chapter 5

GENERAL CONCLUSIONS

5.1. Background

It is generally accepted that the concentration of dust is proportional to seasonal change and droughts in Southern Africa. Therefore one might expect the prevalence of associated airborne microorganisms to follow a similar pattern. Similarly, the distribution and composition of these microbial communities are influenced by environmental conditions that include relative humidity (Venter *et al.*, 2004). In the Free State Province, South Africa, as is the case elsewhere, dust and its associated microbiota get blown into almost any unsealed environment including the food storage facilities of the Primary School Nutrition Program (PSNP). The PSNP, a school-feeding scheme, was introduced nationwide in South Africa in 1994, following President Nelson Mandela's announcement in his State of the Nation Address on 24 May 1994 that such a scheme would be implemented in every primary school where a need was identified (Kloka, 2003). Within the PSNP, food items are delivered to schools by private contractors and further prepared on the school premises by volunteers from the local community. The items vary from province to province and include, amongst other things, products such as fortified biscuits and protein enriched drinks.

As biscuits, fortified with vitamins, proteins and sugar, are stored under sub-optimal conditions prior to consumption in this feeding programme, the

mentioned fortified foods will inevitably be contaminated with dust and associated microbiota. Season-related changes in moisture levels, as well as the occurrence of rain storms, further challenge the ability of the poorly stored hygroscopic fortified biscuits to withstand moisture absorption and subsequently microbial proliferation. Growth of the contaminant bacteria and fungi will in turn not only alter the safety of the products but also their functionality. Therefore, when thus presenting this food to a target consumer who is already malnourished and in several cases immuno-compromised, both an acute and a chronic impact on the health of that consumer may be expected.

With this as background the following questions arose: a) whether the level and distribution of viable airborne microorganisms in the storage rooms and the outdoor environment (in many cases the classroom) at both rural (higher dust exposure) and urban schools participating in the PSNP would pose any health threats and have the possibility of contaminating poorly stored foodstuffs and b) whether the microbial hazards associated with fortified biscuits would be season bound and have the ability to alter the functionality of the fortified biscuits.

5.2. Chapter 1-4

Besides the preservation of the organoleptic property and the integrity of the food, the objective of proper food storage is to protect food from pests and other contaminating elements such as microbes. In an environment where good storage practice is not possible due to a lack in infrastructure, special care regarding the kind of food stored and packaging material used should be taken to ensure food safety. An environment that typifies this is that of schools participating in feeding programmes. From our results it could be concluded that though the storage facilities at the schools in question are, according to all technical data, not suitable for food storage, they did seem to control some of the extrinsic factors that influence microbial viability. No control of these storerooms over the resident airborne bacteria was however noted. The results presented in this study further identified the occupants of the schools as the possible source of the airborne bacteria present in the storerooms and not the building or the surrounding environment, which also calls for food storage in facilities that are separated or isolated from the occupants or packaging material that can be re-sealed. Manufacturers of the food for feeding programmes are further cautioned not to rely solely on the properties of food, such as a reduced water activity, as safeguard, as the inability of the assessed storerooms to control humidity fluctuations could result in the food being unsuspectingly hydrated. It was further noted that, in South Africa, unlike the case in more humid countries, the facility design and geographical localisation had a limited influence on the resident bioaerosol profiles.

In this environment, aerosolised endo- and mycotoxin are expected to be low and thus pose a limited threat to the occupants. The same might not be true for allergies as continuous exposure of the occupants to constant levels of fungi and fungal spores could lead to gradual sensitisation to these allergens. It should further be noted that the occupants of the mentioned schools are probably not knowledgeable regarding proper food storage practice and are probably unaware of the threats posed by the resident microbiota.

It was further evident that both the occupants and the food stored open in its original containers are exposed to several fungal genera that could proliferate on the food (in the presence of moisture) in addition to causing allergies. Moreover, the fungal levels seem more closely correlated to dust levels than to humidity and is probably influenced by the outdoor environment. This explains the influence of locality of the schools on the fungal population profile. Control of the airborne fungal load and population profile in these schools is virtually impossible as the only ventilation available for the occupants is natural moving air. Likewise, the food is exposed to the same contaminants and care should be taken to reseal the food stored (for distribution to the occupants) as this would not only limit the contamination levels but also the exposure of the food to moisture.

This study further showed that the fortified biscuits served to children at primary schools in South Africa are contaminated with microbiota irrespective of the season of manufacture. The type of contaminants further suggests that handling by humans, probably during packaging or re-packaging, is the major source of

contamination. The study further evaluated the ability of the staphylococcal species (osmotolerant) isolated from these biscuits to proliferate once the water activity of the biscuits was increased. A simulation in this regard was performed as poor storage facilities regularly lead to the biscuits being exposed to increased moisture levels as well as to rain (leaky roofs).

From the results it was concluded that during the first two weeks of expose to conditions that favour microbial growth the nutritional value of fortified biscuits decreases in the presence of staphylococci. This does not coincide with an increase in bacterial numbers and does influence the safety of the product (based on staphylococcal counts). The bacteria do however adapt and increase their growth rate after a further twelve days. If consumed after a 21-day period, therefore, biscuits exposed to moisture would be unsafe.

5.3. Recommendations

To verify and address the concerns raised in this study it is recommended that the ability of microorganisms to proliferate on the foods (with different water activities) provided to children in feeding programmes be assessed and the menus adjusted in accordance with season-associated changes in the extrinsic factors that influence microbial viability and growth on the foods. Specific attention should also be given to the development of functional packaging that could be re-sealed after being opened, as was given to the development of functional "microbial unfriendly" foods. In addition, the problem of poor storage practices could finally be dealt with by printing proper storage instructions on the boxes or include these as an insert in the box. Finally, it is important that the volunteer workers receive appropriate training on aspects such as proper food handling techniques and basic food protection principles.

5.4. Future research

Further investigation is required to establish the growth of other (than identified in this study) common bacterial contaminants on the biscuits and their concomitant functional ingredient degradation ability. An evaluation of the impact of functional packaging on the quality and safety of the products served to the children should also be conducted. Similarly, a detailed study on the influence of manufacturing and packaging procedures on the microbial contaminant levels of the food served within the PSNP should be conducted. Finally, a study quantifying the relationship between airborne microbiota and allergy-induced asthma in these school children would shed more light on the impact of bioaerosols on child health.

5.5. References

Kloka, D. 2003. Primary School Nutrition Programme Republic of South Africa. Department of Health. South Africa. 1-4.

Venter, P., Lues, J. F. R. and Theron, H. 2004. Quantification of bioaerosols in automated chicken egg production plants. *Journal of Poultry Science*. 83: 1226-1231.

APPENDIX A

Figure 5.1 The SAS Super 90 microbial air sampler during sampling in the primary school storeroom

Figure 5.2 Fungal colonies on the Potato Dextrose Agar

Figure 5.3 Inspection checklist used to conduct check-up in a storage facility

Figure 5.4 Fortified biscuits sample collection

APPENDIX B

THE INFLUENCE OF STORAGE PRACTICES ON VITAMIN DEGRADATION IN FORTIFIED BISCUITS

District ……………………………………………………….

Name of school ………………………………………… Classification ……………..

Date of inspection……………………………………… Time ………………………

Storage room

Size of storage room ☐

Ventilation: Artificial ☐

Natural ☐

If artificial, specify ………………………………

Natural (Windows): Closed ☐

Open ☐

Air bricks available: Yes ☐

No ☐

Ceiling present: Yes ☐

No ☐

Distance of the ceiling from the ground: ☐

Storage order: Crowded ☐

Organised ☐

Hygiene status: Good ☐

Unhygienic ☐

Items other than the biscuits stored in the storeroom: Yes ☐

No ☐

If yes specify:……………………………………………………..

Temperature: ☐ Humidity: ☐ Light: ☐

Fortified biscuits

Serial number of the batch of biscuits: ☐

Date of arrival: ☐

Provider of the biscuits: ☐

Storage method of the biscuits

Container: Box ☐

Plastic ☐

Paper bag ☐

Plastic bucket ☐

Stainless steel bucket ☐

Other ☐

If other, specify:………………………………………………………..

Placing of the biscuit container: Shelves ☐

Floor ☐

Close to the wall ☐

Far from the wall ☐