

Food safety and quality throughout the apple export chain

by

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DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Master of Science to the University of Pretoria contains my own independent work and has not been submitted for any degree at any other university.

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July 2006

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CHAPTER 1

GENERAL INTRODUCTION

“Access to nutritionally adequate and safe food is a right of each individual.”

(World Health Organization, 1996 from Käferstein, 2003)

For the last two decades, human nutrition research has shown that a well-balanced diet, consisting of satisfactory fresh produce intake, promotes good health and reduction of diseases (Eskin, 1991). Despite benefits related to eating fresh fruit and vegetables, these products can act as transmission vehicles for infectious pathogens, which is of concern for food safety management systems (Meng and Doyle, 2002). The fresh nature of these products, together with subsequent storage conditions and further handling, have presented indigenous and human pathogenic microorganisms with new ecosystems (Francis *et al.*, 1999).

In recent years, man has become preoccupied with transmission of foodborne pathogens so that food safety has become the global minimal standard for international trade (Bracket, 1999). Electron microscopy has revealed the potential of microbial cells to attach to food surfaces, becoming entrapped within the food structure, becoming able to grow as micro-colonies and biofilms (Beuchat, 2002). Foodborne pathogens that are capable of growing or maintaining an infectious potential include *Shigella*, *Salmonella*, *Staphylococcus* spp., enterotoxigenic and enterohaemorrhagic *Escherichia coli* and *Listeria monocytogenes*, amongst the most notable (Beuchat, 2002; Bracket, 1992; Untermann, 1998). Foodborne pathogens cause approximately 76 million illnesses and 5000 deaths each year in the USA alone (Meng and Doyle, 2002). Young children, elderly people and immuno-compromised individuals are most vulnerable to foodborne diseases (De Roeve, 1999).

Postharvest spoilage, which affects the quality of economically important fruit crops, results in reduced shelf life and cause major economic losses to the fruit industry (Marek

et al., 2003). Two principal factors, namely high water content and wounding, make plants more susceptible to spoilage. High water content in fruit creates optimal conditions for pathogen invasion, while wounds inflicted on plant organs during storage, as a result of harvesting and transportation creates an easy infection route (Spadaro and Gullino, 2004).

Current industry practices contribute to global rejection of export consignments due to postharvest decay of fresh produce and the presence of foodborne pathogens. In 1995, fresh fruit and vegetables and dairy products accounted for half the food being lost at the retail level in the USA. According to published studies, a typical food product is handled on average 33 times (by food processors, brokers and wholesalers) before a consumer in the supermarket touches it (Kantor *et al.*, 2004). The cause of many problems in the fruit industry may therefore be traced back to mismanagement of the raw commodity (Downing, 1989). Every step from production through to consumption can have a dramatic impact on the safety and quality of fruit (Beuchat, 1995; Brackett, 1999). Contamination of products may consequently take place at all stages during production and processing. Possible infection sources are soil, faeces, water, ice, animals, handling of products, harvesting and processing equipment, and transport (Johannessen *et al.*, 2002). Infection commonly follows rough handling of fruit and may be acquired through subsequent contact with contaminated surfaces (Arthey and Ashurst, 1996; Downing, 1989).

Environmental storage conditions affect both the final populations and types of microorganisms that will grow on or in fresh produce. Temperature and relative humidity strongly affect the postharvest life of fresh produce. Fresh foods stored or transported at improper temperatures can undergo microbial degradation (Kantor *et al.*, 2004). The length of time fresh produce is held prior to consumption can also influence eventual microbial populations (Brackett, 1992; Madden, 1992).

The domestic apple, *Malus X domestica* Borkh is one of the oldest world fruit crops. It is by far the most important deciduous tree fruit and is affordable. Due to improved storage conditions, apples can be retained up to 12 months or longer (<http://www.bestapples.com/facts/controlled.html>) before releasing the product on to the market. This enables

retailers to more effectively manage stock flow and price control (Jackson, 2003). In South Africa, these opportunities have resulted in major growth for the industry, particularly in terms of its export potential, so that to date 80% of its apples are exported to Europe (Groenewald, 2000).

As apples ripen they become susceptible to attack by a variety of fungi to which they maintained resistant during periods of fruit development on the tree (Dennis, 1983; Arthey and Ashurst, 1996). Important elements of the postharvest environment are humidity, free water and temperature. Most fruit pathogens are fungi and fungal spores require high humidity or free water to germinate (Vismer *et al.*, 1996). Fruit that is kept in a carton box surrounded by a humid microclimate provides an optimal habitat for the pathogen (Arthey and Ashurst, 1996).

Foodborne disease outbreaks have not been related to fresh apple consumption, but to apple juice and cider contaminated with *E.coli* 0157:H7 (Dingman, 2000; Janisiewicz *et al.*, 1999) and *Salmonella* spp. (Miller and Kaspar, 1994; Parnell and Harris, 2003).

Evaluation of the level of microbial contamination in terms of standardized guidelines in places at risk has recently been considered a basic step towards disease prevention (Shintani *et al.*, 2004) and most studies have been carried out in hospitals, healthcare or food-processing facilities (Dancer, 2004; Pasquarella *et al.*, 2000). Since these standards are not of relevance in fresh fruit handling systems, a more realistic standard should therefore be developed.

The purpose of this study was to investigate hygiene standards in fresh fruit handling environments throughout the export chain and safety of apples as they move through the export chain. This study will hopefully set a baseline for developing fresh fruit handling standards.

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CHAPTER 2

LITERATURE REVIEW

FOOD SAFETY AND QUALITY THROUGHOUT THE APPLE EXPORT CHAIN.

1. INTRODUCTION

Global food trade has increased and resulted in new sets of standards and requirements to ensure fair trade (<http://www.codexalimentarius.net>). Some of these new systems are based on food safety assurance and require detailed knowledge of the product, its production and microbial dynamics. It is the aim of this review to highlight the critical factors from production to consumption that can impact on trade.

2. FRUIT EXPORT

Fruits and vegetables play an important role in providing essential vitamins, minerals and dietary fibre in the human diet (Rangarajan *et al.*, 2002; Spadaro and Gullino, 2004). Research has shown that diets low in fat and high in fibre, with at least five servings of fruits and vegetables a day, are protective against many cancers and lessen the risk of heart disease (De Roever, 1999).

Advances in global production and distribution of food have enabled the fresh produce industry to supply most types of fruits to the consumer all year round (Beuchat, 1995). The Food and Agriculture Organisation of the United Nations estimated that the world production of fruits over a three-year period (1993-1995) was 448 million tons. This trend increased, reaching a total global production of 469 million tons in 1996, the most recent figure available (<http://www.fao.org/DOCREP/005/Y4358E/y4385e04.htm>).

The USA dominates international trade in fresh fruits in developed countries, and is ranked number one as importer and exporter, accounting for about 18 % flow in world trade of fresh produce (<http://www.fao.org/DOCREP/005/Y4358E/y4385e04.htm>). Increase in global trade has resulted in more countries moving food products into foreign

markets so that food from 130 countries around the world is available to USA consumers alone (Rangarajan *et al.*, 2002). As a group, the European Union forms the largest player, with 15 additional export and import commodities. Within Europe, Germany is the principal exporter, Spain is the principal supplier; and the Netherlands plays a vital role in the physical distribution of products. In the Southern Hemisphere, South Africa has become one of the major suppliers of fresh produce, mostly fruits (<http://www.fao.org/DOCREP/005/Y4358E/y4385e04.htm>).

3. FRUIT QUALITY

In developed countries, ongoing consumer demand for new fruit varieties has contributed to an increase in trade volume from subtropical regions that mostly represent developing countries. Through the last decade, quality has become an essential feature to ensure market access and effective competition from an ever-growing number of exporting countries (Luning, *et al.*, 2002). Quality is defined as those characteristics of food that lead to consumer satisfaction (Harker *et al.*, 2003; Shewfelt, 1999) and include sensory properties, shelf life, product safety, appearance and nutritional value (Eskin, 1991; Luning *et al.*, 2002).

Variability in quality arises during production, harvesting, postharvest handling, packinghouse operation, distribution, delivery, and marketing (O'Hare *et al.*, 2001). The fresh produce handling chain involves a great number of stakeholders each with their own ideas and practices (O'Hare *et al.*, 2001). Therefore, in order to obtain a satisfactory end product, general practices should be managed along the whole food chain from the producer to the consumer (Luning *et al.*, 2002).

Quality of fruit is influenced, amongst other factors, by its microbiological flora (Brackett, 1992; European Commission, 2002). Those factors that affect the dynamics of growth of microorganisms on food include intrinsic (substrate properties), external (temperature, relative humidity, gas composition), processing (impact of the environment) and implicit (initial dominant microflora) properties (De Roever, 1999; Madden, 1992; Mossel *et al.*, 1995).

Unlike most other foods, fruit after picking and before processing is physiologically active and in a senescent phase of its life (Frazier and Westhoff, 1988). Fruit carry a natural non-pathogenic epiphytic microflora with the majority of bacteria being Gram-negative and belonging either to the *Pseudomonas*-group or to the *Enterobacteriaceae* (European Commission, 2002). During growth, harvest, transportation and further handling, the produce can become contaminated with other microorganisms from various sources (De Roever, 1999; European Commission, 2002). These contaminants may be either plant pathogens that cause fruit decay and eventually loss (Marek *et al.*, 2003), or they may be human pathogens that are accidentally introduced and attach to the fruit surface, potentially resulting in a food safety risk (Beuchat, 1995).

Contaminant sources include soil, faeces (both of human and animal origin), water (irrigation and cleaning) (Johannessen *et al.*, 2002), air (Mainelis *et al.*, 2002), ice, animals (including insects and birds), handling of products, harvesting and processing equipment, and transport (Johannessen *et al.*, 2002). Infection commonly follows rough handling of fruit and may be acquired through subsequent contact with contaminated picking bags or receptacles, boxes, bins, wash water or sorting equipment (Arthey and Ashurst, 1996; Downing, 1989).

Storage conditions (temperature, relative humidity, holding time) also affect both the final population and types of microorganisms that will grow on fresh fruit. In an article of Eurofruit magazine (2002), it is mentioned that vessel delays at Cape Town port are sometimes between 27-40 hours and the worst-case scenario recorded was 100 hours. Ineffective cold chain management further contributes to loss of product quality, impacts on microbial growth and survival and may result in contamination of produce (Brackett, 1992; Madden, 1992; Madden and Joseph, 1993). Microorganisms grow best at optimum temperatures, but they may also be able to grow near their maximum or minimum temperature ranges (Wiley, 1994). Some foodborne pathogens like *Listeria monocytogenes*, for example, can grow at refrigerated temperatures of 4°C (Beuchat, 1995). Concerning humidity, the highest humidity, nearest saturation, is required for most bacterial growth; less moisture is required by yeasts (90 to 92 %), and even less by fungi, which can grow in a relative humidity of 85 to 90 %. Changes in humidity and

temperature during storage may cause precipitation of moisture on food surfaces, favouring microbial spoilage (Frazier and Westhoff, 1988).

The low pH of apples and apple products restricts the growth of a wide variety of microorganisms, so that only yeasts, fungi, lactic- and acetic acid bacteria are capable of prolific growth and survival (Downing, 1989) (Table 1). Teixidó *et al.* (1998) did a study on the natural microbial population dynamics of Golden Delicious apples and found that the frequency of bacteria isolated was less than 5% of the total microflora, and that bacteria were seldom isolated from fruit kept in cold storage. This could probably be due to partial sensitivity of non-psychrotrophic species to low temperature (-1° to 4°C) conditions during cold storage. Yeasts were the dominant colonizers, especially after two months of cold storage; this was followed by the presence of fungi (Teixidó *et al.*, 1998). Individually, healthy apples carry a fungal load of 10^3 to 10^5 organisms per apple, while higher counts normally indicate infected or damaged fruit (Vismer *et al.*, 1996).

4. THE APPLE EXPORT CHAIN

The domestic apple, *Malus domestica* Borkh., is believed to originate from *M.sieverssii* (Ledeb) M.Roem., a diverse species from the mountains of central Asia (Jones and Aldwinckle, 1990). Apple cultivars produced in South Africa for export include Royal Gala, Pink lady, Fuji, Big Chief, Top Red, Sundowner, Braeburn, Granny Smith and Golden Delicious (Jooste, 2000). In 2000, it was estimated that roughly 80 % of South Africa's apple production is exported to Europe (Groenewald, 2000). The export market has expanded tremendously over the past few years, with apples being one of the fastest growing agricultural export commodities (Dall, 1999). In 2002, South Africa was the 4th largest producer of apples in the Southern Hemisphere, with a total production of 579 257 metric tonnes cultivated on a total area of 22 454 hectares (Anon, 2002).

Table 1 Major microbial species isolated from apple fruit surfaces (compiled from Downing, 1989; Leibinger *et al.*, 1997; Penrose *et al.*, 1989; Sholberg and Haag, 1996; Teixidó *et al.*, 1999)

Type of microorganism	Species
Yeasts	<i>Candida pulcherrima</i> (Lindler) Windisch
	<i>Candida</i> sp.
	<i>Torulopsis</i> sp.
	<i>Rhodotorula</i> sp.
Moulds	<i>Penicillium expansum</i> Link (blue mould rot)
	<i>Monilla</i> sp.
	<i>Mucor</i> sp. (Mucor rot or stem end rot)
	<i>Cladosporium</i> sp.
	<i>Botrytis cinerea</i> Persoon:Fries (gray mould rot, dry eye-rot)
	<i>Cryptosporiopsis</i> sp. (bull's-eye rot)
	<i>Alternaria</i> sp. (Cottony mould, fruit spotting & core-rot)
	<i>Butlerelfia</i> sp. (fish-eye spot)
	<i>Pleospora herbarum</i> Fries:Fries (surface rot of apples subjected to injury from freezing)
	<i>Gleosporium perennans</i> Zeller & Childs (apple rot)
Bacteria (isolated from apple juice)	<i>Lactobacillus</i> sp.
	<i>Leuconostoc</i> sp.
	<i>Acetobacter aceti</i> subsp. <i>Xylinum</i> (Brown) De Ley & Frateur
	<i>Gluconobacter</i> sp.

In order to meet export requirements, an apple should have good quality. Factors that contribute to its final quality include the following:

1. Preharvest conditions and harvest factors (genetically controlled factors like cultivar and strain, climatic conditions, soil conditions, agricultural practices and harvesting practices)
2. Postharvest factors (physiology, wounding, pathology and cold chain management).

Since the postharvest environment has a great influence on product quality, each step in the export process should be analysed properly to determine areas of concern. In South Africa, apples from the orchard are handpicked into bags, transferred to field bins and transported to a packing facility either on or off farm. Here they are pre-cooled overnight to remove field heat, after which they are moved to the packinghouse for grading, packing and pallet stacking. Final cooling to export temperature ($-0.5 \pm 0.5^{\circ}\text{C}$) (<http://www.ppecb.co.za/community/procedures>) of the pallet loads occurs after dispensation. Once apples have reached the export temperature they can be loaded into cooled trucks for transport to one of the destination ports (Figure 1). Here the fruit is cool-stored for a limited period, containerised and loaded onto a vessel that transports it to a predetermined export country. Only after the apples have been distributed and repacked, do they reach the supermarket.

5. PLANT PATHOGENS ON FRESH PRODUCE

Postharvest diseases are among the most important causes for losses and reduction of fruit quality (Kampp, 1994). It is estimated that between 49-80% of export fruit and vegetables reach the consumer, while the difference is lost during the various steps from harvest to consumption (<http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>). The consumer is generally unaware of all the factors impacting on quality and causing losses. Aspects such as separating decayed fruit from healthy ones in the orchard or packinghouse, the cost of repacking, and the accumulated cost of investment in harvesting, packing and shipping of the decayed product on the export markets greatly

exceeds the value of the raw commodity in the field before harvest (Haard and Salunkhe, 1975).

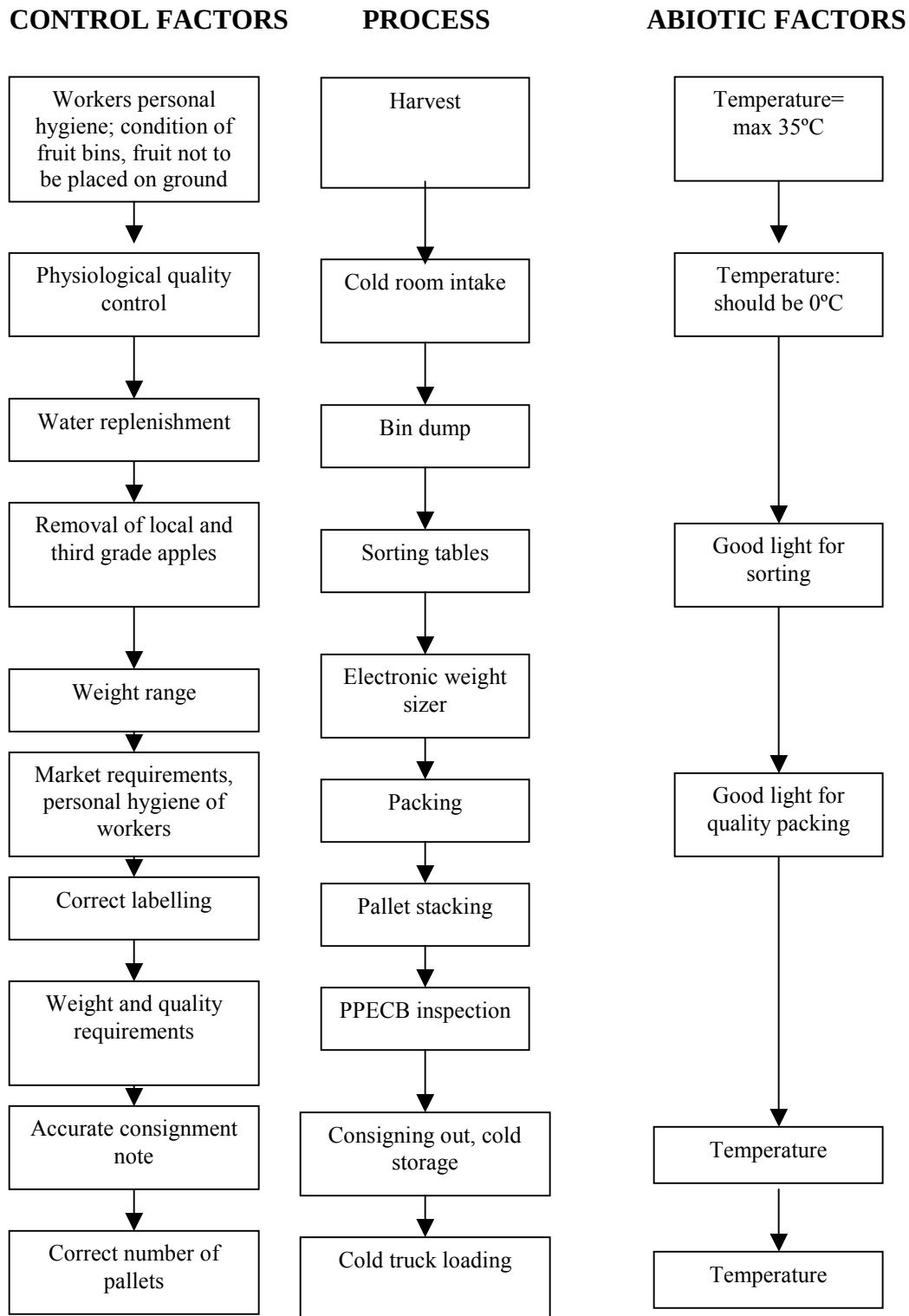


Figure 1 Apple export flow diagram for farms

The export environment has a great influence on maintaining product quality and reducing postharvest losses (Evans, *et al.*, 2004). For a postharvest disease to occur and develop, a combination of three factors should be present: a susceptible plant, an infective pathogen, and a favorable environment. Changes in environmental conditions may drastically influence the development of a disease in progress or the beginning of a new disease (Agrios, 1997). Important elements of the postharvest environment are humidity, free water, temperature (<http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>) and to a lesser extent, light and pH (Agrios, 1997). The inherent low pH of most fruits favours predominance by moulds (Frazier and Westhoff, 1988) so that most of the postharvest fruit pathogens are fungi (<http://www.fao.org>), and fungal spores require high humidity or free water to germinate (Vismer *et al.*, 1996). Fruits that are kept in a confined space (like a box) are surrounded by a humid microclimate; and can therefore provide an optimal habitat for plant pathogens (Arthey and Ashurst, 1996).

The export environment can also serve as an inoculum for plant pathogens. Pathogenic organisms may be found on the floors, walls and in the drains of facilities or on equipment surfaces used for sorting, grading and packing (FDA, 1998). Microbial loads on surfaces and equipment again, vary in different food plants depending on the microbial quality of the food and the cleaning programs in operation (Evans, *et al.*, 2004).

According to Vismer *et al.* (1996), the most important source of the microflora of apples is derived from the orchard: The natural microflora are those adhering to the surface of the fruit and they tend to be stable. Contaminating microflora are derived from external vectors such as rain, soil, dust, irrigation, insects, bird and rodent contamination deposited on the surface of the fruit (Frazier and Westhoff, 1988; Vismer *et al.*, 1996). Most microorganisms are unable to penetrate the waxy skin of apples, but wounding caused by handling and sorting can facilitate entry of these microorganisms (Vismer *et al.*, 1996). Also, as apples ripen, they become susceptible to attack by a variety of fungi to which they were resistant during their period of development on the tree (Arthey and Ashurst, 1996; Dennis, 1983).

Fungal pathogens are the main cause of postharvest losses of apples that can be as high as 50 % (Spadaro *et al.*, 2004). Initial contamination sources include flume and sorting

water, unclean storage crates (Spotts and Cervantes, 1993, Vismer *et al.*, 1996) or storage facilities (Vismer *et al.*, 1996). Although more than 90 fungal species have been described to cause apple decay during storage, blue mould caused by *Penicillium* spp. seems to be the most important postharvest disease worldwide (Jones and Aldwinckle, 1990). *Penicillium expansum* is a wound pathogen and has been shown to produce patulin, a mutagenic, immunotoxic, and neurotoxic mycotoxin (Pianzzola *et al.*, 2004).

Postharvest fungi represented 71 % of the total microorganisms isolated from all components (including sorting tables, sizer cups, flumes, etc) in packing and storage facilities (Combrink *et al.*, 1994). *Penicillium* and *Mucor* spp. were the dominant postharvest fungi recorded in several apple packinghouses in South Africa and their propagules were present everywhere, except in chlorinated flume water. The effect of the chlorine wash had no impact since fruit became contaminated again once it passed over dirty sizer cups and packing tables (Combrink *et al.*, 1994).

Wherever water comes into contact with fresh produce, its quality determines the potential for pathogen contamination (FDA, 1998). Washing water can easily spread contaminants over produce, intensifying the potential for microbial spoilage. In a study performed by Sholberg and Haag (1996), defrosted cooling water, originating from packinghouses, was believed to serve as a source of inoculum for fungi like *Cladosporium* spp., *Penicillium* spp. and *Phialophora fastigiata* Lagerberg & Melin. A positive relationship between decay and spore populations in immersion water has been shown for apples in the USA and Canada (Sholberg and Haag, 1996). Refrigeration units used for controlled atmosphere by most of the apple packinghouses are de-iced and washed daily with warm water pumped from a central defrost tank. The tank can hold up to 6000 litres of water that is also used as coolant for each room's compressor. This water is recycled during the winter months. Pathogens could accumulate in the defrost water and be dispersed by the forced air fans onto the apples and bins in the storage rooms (Sholberg and Haag, 1996).

Studies by Sholberg and Haag (1996) indicate that type of cultivar also seems to have an effect on the occurrence of pathogens on the fruit surface (Table 2). Apple cultivars were

sampled over a period of three years. The cultivar most often observed with fungal infections was McIntosh, followed by Spartan, Red Delicious, and Golden Delicious (Sholberg and Haag, 1996).

Table 2 Relative incidence* of fungi isolated from diseased apples of several cultivars sampled from British Columbia packinghouses (Sholberg and Haag, 1996)

Apple cultivar	No. of fruit infected	Pathogens isolated*				
		<i>Botrytis cinerea</i>	<i>Cryptosporiopsis curvispora</i>	<i>Mucor</i> spp.	<i>Penicillium</i> spp.	Miscellaneous
-Gala	24	41.7	0.0	8.3	45.8	4.2
-Golden Delicious	56	12.5	46.4	12.5	16.1	12.5
-Jonagold	21	0.0	0.0	47.6	28.6	23.8
-McIntosh	225	37.8	8.9	16.9	22.7	13.8
-Red Delicious	62	38.7	0.0	16.1	33.9	11.3
-Spartan	108	48.1	0.0	16.7	25.0	10.2

*Incidence is expressed as the percentage of diseased apples from which each fungus was isolated.

6. HUMAN PATHOGENS ON FRESH PRODUCE

The surfaces of food products that are exposed to contagious environments or are kept within improper storage conditions are more susceptible to microbial contamination. These food products may harbour foodborne microorganisms or promote their growth that can lead to foodborne illness (Yand and Irudayaraj, 2003). Therefore, despite benefits related to eating fresh fruit, the product can act as a transmission vehicle for infectious pathogens, which is a major concern within food safety management systems (Meng and Doyle, 2002). Foodborne diseases cause approximately 76 million illnesses, 325 000 hospitalisations, and 5000 deaths each year in the USA alone (Meng and Doyle, 2002), while 3-8 % of gastrointestinal illness outbreaks are traceable to consuming fruits and vegetables (Madden, 1992). Young children, elderly people and immuno-

compromised individuals are most susceptible to these diseases (De Roever, 1999), an “at-risk” group that comprises approximately 20 % of the population (Beumer and Kusumaningrum, 2003).

In time, consumer attitudes towards food products have changed. In the late 1970’s, price was the major determinant in food purchases. In the early 1980’s, visual quality and the perception that products were well kept and attractively presented were the overall determinants. Fast foods, minimally processed products, frozen goods and ready-to-eat meals, dominated the consumer trends by the late 1980’s. Only from the 1990’s to the present, has there been increasing concern over food safety issues such as pesticide residues and microbial contaminants, sanitation of the work environment, personal hygiene of the workers, environmental protection and human welfare (O’Hare *et al.*, 2001).

Data from the Centres for Disease Control and Prevention from 1973 to 1992 suggest there has been a doubling in the annual number of reported foodborne outbreaks (De Roever, 1999). This increase is primarily due to increasing complexities of the food system, aging of the human population, increased appearance of chronic illnesses and compromised immunity, changing awareness of hygiene and associated risks and adaptation of microorganisms (Beuchat, 1995; De Roever, 1999).

According to Lammerding and Fazil (2000), the four cornerstones of microbial food safety risk assessment are hazard identification, exposure assessment, hazard characterization, and risk characterization. These four steps represent a systematic process for identifying consequences and their probabilities arising from consumption of foods that may be contaminated with microbial pathogens. A hazard refers to a biological agent (microorganism or its toxin) that has “the potential to cause an adverse health effect”. Hazard identification identifies the issues of concern, while exposure assessment generates estimates of the likelihood and magnitude of exposure to the hazard. This sets the stage for the next two steps in which the exposure outputs are translated into a measure of risk (Lammerding and Fazil, 2000; Van Schothorst, 2002).

In contrast to fruit quality problems that are normally caused by fungal plant pathogens (<http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>), foodborne pathogens of greatest concern are mainly bacterial species (Beuchat, 1995) that do not cause fruit decay but are a human health risk. These pathogens include *Shigella*, *Salmonella*, *Staphylococcus* spp., enterohaemorrhagic *Escherichia coli* and *Listeria monocytogenes*, which represent the major public health concerns (Beuchat, 2002; Brackett, 1992; Untermann, 1998). These pathogens have been isolated from various fresh products and have been correlated with disease outbreaks and illnesses (Table 3).

Although no foodborne outbreaks have been linked to the consumption of fresh apples (Janisiewicz, *et al.*, 1999; Kenney and Beuchat, 2002), *Escherichia coli* 0157:H7 and *Salmonella* spp. infections have been epidemiologically linked to the use of unpasteurized apple juice and cider (Kenney and Beuchat, 2002). For long, it was believed that the low pH of apple juice would restrict the growth of human pathogens, but research has shown that *E.coli* 0157:H7 and *Salmonella* spp. are able to survive in fruit juice for several days (Kenney and Beuchat, 2002; Miller and Kaspar, 1994). In 1996, four outbreaks of foodborne illnesses related to contaminated unpasteurized apple cider were reported (Besser *et al.*, 1993; Dingman, 2000). These included an outbreak caused by *E.coli* 0157:H7 which resulted in the death of a 16-month-old child (Besser *et al.*, 1993). Potential sources of *E.coli* are numerous, and include bird droppings, fruit flies, house flies (Dingman, 2000; Janisiewicz *et al.*, 1999), faeces of domestic and wild animals (Janisiewicz *et al.*, 1999) and handling by food handlers following poor hygiene standards (Du *et al.*, 2003).

Table 3 Summary of foodborne pathogens isolated from fresh produce and the respective disease symptoms they cause

Organism	Sources	Product	Infection symptoms	Reference
<i>Escherichia coli</i> 0157:H7	Animal and human faeces, irrigation water, food handlers	Mixed vegetables, lettuce, apple cider, apple juice, orange juice, celery, coriander	Severe bloody diarrhoea, abdominal pain, haemolytic uraemic syndrome (young children), thrombotic thrombocytopenic purpura	Beuchat (1995); European Commission (2002); Kenney and Beuchat (2002); Miller and Kaspar (1994); Nguyen-the and Carlin (1994); Padhye and Doyle (1992); Untermann (1998)
<i>Salmonella</i> spp.	Sewage, manure, bird droppings, water	Celery, watercress, watermelon, lettuce, cabbage, endive, berries, apple, apple juice, orange juice, artichoke, bean sprouts	Gastrointestinal illness (Diarrhoea, fever, abdominal cramps, vomiting)	European Commission (2002); Geldreich and Bordner (1971), Kenney and Beuchat (2002); Nguyen-the and Carlin (1994)
<i>Shigella</i> spp.	Food handlers, water, manure	Cabbage, onion, garlic, lettuce, papaya, watermelon	Shigellosis (abdominal cramps, fever, diarrhoea), gastroenteritis	Beuchat (1995); European Commission (2002); Geldreich and Bordner (1971); Madden (1992); Nguyen-the and Carlin (1994)
<i>Staphylococcus</i> spp.	Food handlers (nose, saliva, hands)	Cabbage, onion, garlic, lettuce, mushrooms, carrots, parsley, radish	Nosocomial infections	Beuchat (1995) Lengeler <i>et al.</i> (1999); Nguyen-the and Carlin (1994)
<i>Listeria monocytogenes</i>	Vegetation, soil, sewage, decaying plant material (considered ubiquitous)	Chicory salads, cabbage, lettuce, potato, radish, asparagus, broccoli, cauliflower, watercress	Listeriosis, gastroenteritis (fever, muscle aches, nausea), septicaemia, abortion, encephalitis	European Commission (2002); Fenlon (1985); Nguyen-the and Carlin (1994)

Every step in the fresh produce chain has an effect on the microbial status of fresh produce and can be a potential source of contamination (Figure 2) (Beuchat, 1995). The lack of a lethal treatment at any step between farm production and consumption means that pathogens introduced at any point may be present when the produce is consumed (De Roever, 1999). Starting at the producer, the history and location of the land on which the produce is grown is of utmost importance. Fields with low growing crops, on which livestock or wild animals grazed, are more likely to be contaminated with enteric pathogens (Brackett, 1999; De Roever, 1999). A history of flooding can also affect the bacteriological quality of croplands. Floodwater can become polluted with animal wastes and carry contaminants downstream, where they may flood croplands (Brackett, 1999). Epidemiological investigations indicated that diseases associated with fresh fruits and vegetables are primarily those transmitted by a faecal-oral route (De Roever, 1999).

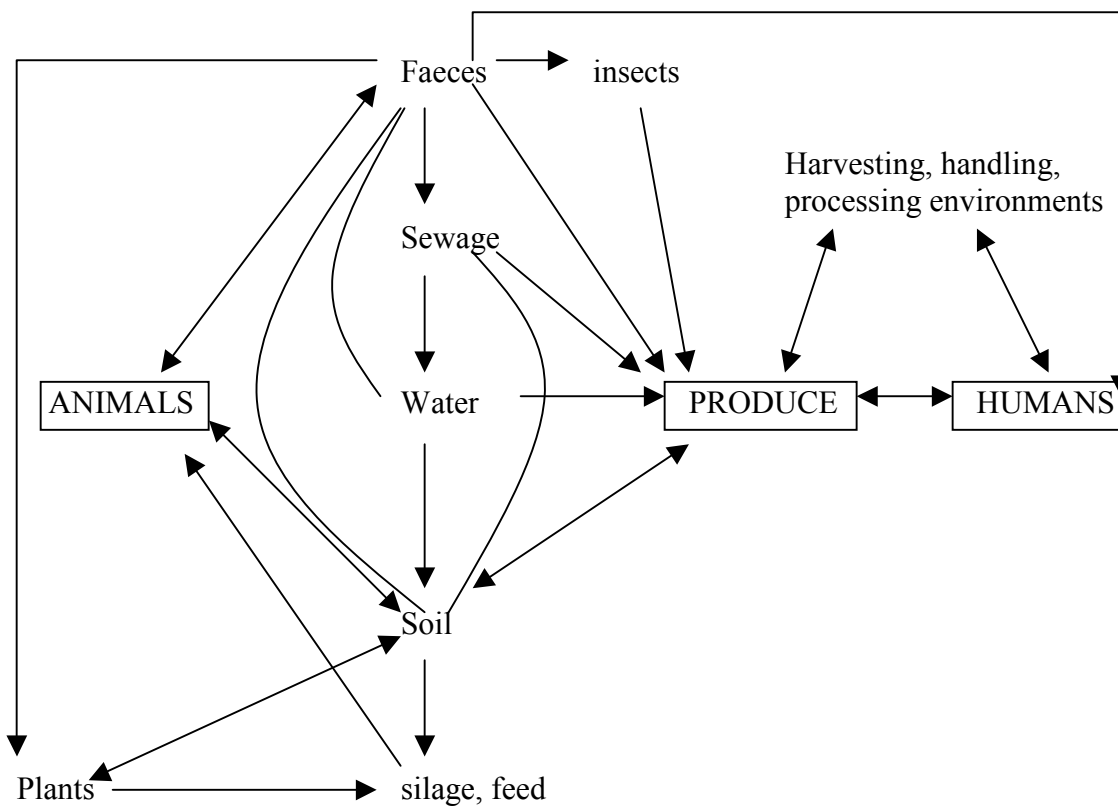


Figure 2 Mechanisms by which fresh produce can become contaminated with pathogenic microorganisms (Beuchat, 1995, adapted).

Another factor influencing the microbiological safety of produce is the type and quality of irrigation water used and its source of origin. Overhead irrigation and wastewater have a high probability of containing pathogenic microorganisms that can potentially contaminate produce (Brackett, 1999). The greatest contamination risk crops in this case are mostly leafy vegetables that provide large surface areas with surface features that promote attachment or entrapment of microorganisms (De Roever, 1999). For fresh commodities like apples and tomatoes, the temperature of the washing water should be higher than that of the produce, or the pressure differential will result in infiltration of bacteria into the plant material (Kenney and Beuchat, 2002).

Food handlers' personal hygiene has resulted in a food safety concern, because they often come from diverse cultural backgrounds with different levels of training. The employees of companies who refuse to enforce basic hygienic practices (Engel, 1998), such as handwashing, are likely to contaminate the produce they touch with foodborne pathogens (Brackett, 1999), for it has been reported that humans shed about 1×10^3 to 1×10^4 viable microorganisms per minute (Frazier and Westhoff, 1988). A study conducted in the Finnish Food Industry, found that some of the most important causes of the spread of foodborne diseases were caused by poor hygiene practices used by food handlers. Although maintenance personnel working in the food industry did not actually touch the products, they touched numerous surfaces that came in contact with unpacked products, like hammers, screwdrivers and spanners (Aarnisal *et al.*, 2003).

7. EXPORT REQUIREMENTS AND QUALITY STANDARDS

One of the main concerns of national governments is that imported food from other countries should be safe and not endanger the health of consumers (<http://www.codexalimentarius.net>). Importing countries therefore set the standards that potential trade partners must meet in order to prevent human illness (<http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>). In the area of food and plant control, these measures could create barriers to international food trade (Stewart *et al.*, 2002; <http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>)

Historically, food safety issues had been developed and dealt with as the need arose in the production of food products. Default values, which represent worst-case scenarios, were derived for the development of processing parameters. These default values were then accepted as regulatory requirements and while some of them have been re-evaluated, the review process lacks scientific-based information (Stewart *et al.*, 2002). Debate over food safety has been prominent in Europe. Here, the use of import bans and intervention by the European Commission is increasingly justified under the precautionary principle. This suggests that regulatory action against risk be taken, even when science has not established direct cause and effect relationships (Otsuki *et al.*, 2001).

No microbial food safety standard for fresh apples exists, but the international guideline for the maximum allowed *E.coli* for fresh-cut vegetables and fruit is less than 100 *E. coli*/g, and for *L. monocytogenes* is less than 10 *L. monocytogenes*/g (Food Science Australia, 2000). A parameter used for *S. aureus* is 100-1000 colony-forming units (cfu)/g whilst for *Salmonella* spp., they should be absent in 25 g of the product (Stekelenburg, 2001).

In a study performed by Berdegué *et al.* (2003), the general primary quality standard imposed by supermarket chains is related to appearance and size. Concerning microbial quality, the Japanese government defines foods with aerobic bacterial plate counts of less than 5 log cfu/g as being safe for consumption (Kaneko *et al.*, 1999) while according to Nguyen-the and Carlin (1994), product quality is often still acceptable with mesophilic bacterial counts of 10^3 to 10^9 cfu/g. Rather than high microbial populations being responsible for deterioration of fresh produce, deterioration may be responsible for high microbial populations, as stated by Zagory (1999). If fruits are handled roughly or held at abusive temperatures, it matters little if they started with 10^2 or 10^6 cfu/g (Zagory, 1999). The maximum acceptable index level of microbial air contamination is 22 cfu/m³ for food industries (Pasquarella *et al.*, 2000) and the US Department of Agriculture has used total microbial counts of less than 5 cfu/cm² in evaluating cleaning efficacy (Dancer, 2004).

In terms of quality parameters, there are three classes of apples, namely “Extra Class”, “Class 1” and “Class 2”. Depending on the class, apples are only exported if they meet

the standards and requirements as stipulated by the Agricultural Product Standards Act of 1990 (South African Department of Agriculture). Before fruit leave the packinghouse of a farm, all pallets are inspected by a PPECB (Perishable Products Export Control Board) inspector to determine whether it meets export standards. These standards require that fruit should be of good quality. Quality factors include shape and colour, presence of hail marks, bruises, blemishes, sunburn, ripeness, chemical residues, to name a few. Each cultivar and each class has different requirements and these should be met according to this Act. Containers and pallets in which apples are packed should be clean, dry and undamaged; should not impart a foreign taste or odour; be free from any visible signs of fungus growth; be strong and rigid so that they don't break or tear to the extent that the apples are damaged during storage or transport (Agricultural Product Standards Act, 1990).

8. CONCLUSION

This review covered topics ranging from decay to safety to product quality, and provides a baseline understanding of the complexity of issues within the fruit trade sector. Several infection points have been identified from production to consumption and these need to be managed within basic good agricultural practices and food safety systems to ensure ultimate safety and quality. Uniform hygiene and specific safety standards for the fresh fruit sector are required and should be well grounded by thorough scientific research.

The review provides background information for this dissertation that covers topics such as hygiene standards; air quality and fruit safety and quality in the apple export chain.

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CHAPTER 3

DETERMINING HYGIENE LEVELS IN THE FRUIT CHAIN ENVIRONMENT FROM SOUTH AFRICA TO EUROPE

ABSTRACT

The quality and safety of fruit depends on every step in the export chain from production through to consumption. Hygiene levels for fruit export environments were determined by sampling two farms and a harbour in South Africa and two harbours, two repacking facilities and two retail centres in Europe. In this first study of its kind, all surfaces that were sampled exceeded the international standard of $<5 \text{ cfu/cm}^2$ for cleaning efficacy. Washing of containers on the harbour in South Africa did not have a significant impact on microbial populations. High microbial counts are often attributed to poor hygiene practices implemented by farmers, while the rest of the distribution chain might also play a significant role in terms of introducing pathogens through unhygienic practices.

1. INTRODUCTION

It is estimated that a quarter of all produce harvested is spoiled before consumption (Frazier and Westhoff, 1988). The eventual cause of many problems in the fruit industry may be traced back to mismanagement and negligence of the raw commodity (Downing, 1989) so that every step from production through to consumption can have a dramatic impact on the safety and quality of fruit (Beuchat, 1995; Bracket, 1999).

Contamination of fresh produce may take place at any of the activities during production and processing. As soon as fruits are collected into bags, bins, boxes or trucks during harvesting, they are subjected to contamination with microorganisms from each other and from the containers. During transportation, mechanical damage may increase susceptibility to decay and growth of microorganisms may take place (Arthey and Ashurst, 1996; Downing, 1989; Frazier and Westhoff, 1988; Johannessen *et al.*, 2002). Throughout the rest of the distribution and export process, build-up of microbial

populations on any equipment or surfaces as the result of microbial growth in the exudates and residues from fruits, may greatly influence the amount of contamination of the foods and the growth of the contaminants (Frazier and Westhoff, 1988). Further handling occurs when fruit arrives at its fore last destination, the wholesale and retail market. Here it is unpacked, sorted and repacked for distribution and sold to the consumer (Geldreich and Bordner, 1970). Many produce managers fail to understand the need for maintaining high sanitation standards and uninterrupted cold storage (Beuchat, 1995).

Farmers are normally blamed for poor hygiene practices, while proper sanitation at all levels in the fruit export chain is crucial (Beuchat, 2002; De Roever, 1999). This includes the avoidance of the use of untreated manure as fertilizers, proper sanitary systems and handwashing facilities for fruit handlers, the use of clean equipment and transportation vehicles and good hygiene in the distribution environment and kitchen (De Roever, 1999). It is therefore important to investigate the parameters that influence the final fruit product. Although no standard has been set for handling fresh fruit and the environments it is exposed to, international standards specify that microbial counts on food-processing equipment should be $<5 \text{ cfu/cm}^2$ in evaluating cleaning efficacy (Dancer, 2004).

The aim of this investigation was to determine hygiene levels of fruit chain environments in South Africa and Europe in order to identify areas of concern in maintaining high fruit quality. Since the environments monitored in this study were not food manufacturing premises where fruit is processed, hygiene standards should be scientifically based and adopted to suite fresh fruit handling facilities.

2. MATERIALS AND METHODS

Sampling was performed at two farms and one harbour in South Africa and at two harbours, two repacking facilities and two retail stores in Europe.

2.1 SWAB SAMPLING

2.1.1 Farms

Transport swabs (MW & E, UK) were taken from five fruit pickers' and five fruit packers' hands, five picking bags, five fruit bins, five bin-liners, five wooden pallet bases, three wall and three floor surfaces in the cold storages, six wall and six floor surfaces in the packing house, five empty unused boxes, five pulp trays, four rollers, four brushers, four flaps, four conveyer belt surfaces, four fruit sizers, four table surfaces, three air-conditioning system surfaces and five bathroom basin tap surfaces in and around the packing house. Specimens were obtained by rubbing an area of 5 cm² with a swab (Rampling *et al.*, 2001) while number of sampling was based on facility size or surface area, and total volume of work to be done in a specified period.

2.1.2 Harbours

Transport swabs were taken from five containers, three cold storage facilities and five trucks at three representative fixed points. These points consisted of floors and walls, but where applicable, air-conditioning systems, silicon fasteners, flaps and plastic sheets were also sampled. At South Africa's harbour, containers were sampled before and after washing. Specimens were obtained as described above. At South Africa's port and one of the European harbours an available ship that had just off loaded its fruit cargo, was also sampled. Three swab specimens were taken from floors and walls respectively on three levels in the ships. Sampling at the South African harbour was done twice for all the areas, except for the reefer ship.

2.1.3 Repacking facilities

Transport swabs were taken from three cold rooms and the packing area at each repacking facility, but where necessary, extra sampling points were selected. Extra points included five packers' hands, five empty unused boxes and five pulp trays. In the cold rooms, three replicates were taken from floors and walls, as described earlier. In the packing areas four replicates were performed.

2.1.4 Retail centres

The holding facility, display area (floors and walls) and five customers' hands were sampled as discussed earlier.

2.2 SWAB SAMPLE PROCESSING

All samples were transported in cooler boxes and processed promptly. The transport swab of each replicate was inoculated in test tubes with nine ml Ringer's (Merck, Johannesburg) solution for a maximum period of 30 minutes. The solution was mixed thoroughly using a vortex shaker (Labotec, South Africa) and 100 microlitres of the solution was spread plated onto Standard 1 Nutrient Agar (STD1) (Biolab, Johannesburg) and Malt Extract Agar (MEA) (Biolab) plates. Three dilutions were performed i.e. 10^{-3} , 10^{-4} and 10^{-5} . The STD1 plates were incubated for 48h at 25°C and MEA plates for 96h at 25°C. Total counts and specie diversity indexes (number of different colony types) were recorded and pure cultures were made only from dominant representative isolates (species predominating at each sampling point throughout the export chain). For the MEA plates, only *Penicillium* spp. were isolated for further identification, because of its association with fruit quality (Agrios, 1997). However, all predominant bacterial species were isolated from STD1 plates, since any of the isolates could be a potential foodborne pathogen. All pure cultures were preserved by freeze-drying (Dhingra and Sinclair, 1985). The identity of bacterial organisms was based on colonial appearance, cellular morphology, motility, Gram stain, oxidase and catalase reactions as appropriate. Confirmation of the bacterial isolates were performed using the API CH, API Staph, API 20E, API 20NE (Biomérieux, France) and Biolog GP2 (Biolog, USA) systems. Identification of fungi was done with the assistance of R. Jacobs, B. Demoz and M. Truter (Plant Pathology laboratories, Mycological Unit, University of Pretoria).

2.3 ENVIRONMENTAL MONITORING

Temperatures (Extech, USA) were measured throughout the experiment with a hygro-thermometer. The measurements were made by keeping the thermometer probe

vertically upright for 30 seconds in the centre of the area analysed. Three readings were made per area analysed.

2.4 STATISTICAL ANALYSIS

Before submitting all data for statistical analysis, two methods were compared to calculate the number of microorganisms per millilitre, since each sample had three counts for the three dilutions performed: one method made use of procedures followed by the South African Bureau of Standards (ISO 4833: 1991), while the other method involved manual filtering where counts for the dilution with the most countable result was used for further analysis. Both of the methods were submitted for statistical analysis, and it was the manual filtering method that produced the most reliable results.

As microbial counts are not normally distributed with heterogeneous variances, differences between treatment effects (areas of sampling) were tested using the method known as generalized linear modelling (Dobson, 1983). The gamma distribution best modelled the data obtained in this study (Lefkovitch, 1993). Fishers' protected t-test Least Significant Difference (LSD) was applied to separate means at the 1 % level of significance. In the case of bacteria, numbers greater than 300 and for fungi and yeasts, numbers greater than 200, were pre-processed before analysis as these censored observations were replaced by estimated values, using the method outlined by Taylor (1973). This method estimates the expected value of each censored observation iteratively conditional on the fact that the value must be greater than the fixed bound, and using the relevant information from the other observations in the experiment.

The aim of the statistical procedure was to identify particular trends in microbial counts and diversities throughout the export chain, and not to determine whether significant differences were present (standard deviations and significance levels are shown in the Appendix and not indicated on graphs).

3. RESULTS

In total approximately 4000 swab samples were analysed for the chain study. Of these, thirty-seven predominant representative bacterial groups and eight *Penicillium* spp. were isolated and identified (Table 1).

3.1 FARMS

Overall, bacteria dominated microbial communities on both farms that were investigated, while fungi and yeasts had lower counts (Fig. 1; APPENDIX B). Average total counts were generally higher for farm nr.1 compared to farm nr.2. Concerning species diversity, microbial diversities were the lowest for the sizers of farm nr.1 and unused boxes for farm nr.2, while hands and air-conditioners generally yielded the highest diversities (Fig. 1 and 2; APPENDIX B).

3.2 HARBOURS

At South Africa's harbour, washing of containers had no significant impact on average microbial total counts or diversities (Fig. 3 and 4; APPENDIX C). Walls yielded the lowest microbial counts for unwashed and washed containers (Fig. 3; APPENDIX C). Bacteria dominated counts, while fungi and yeasts dominated diversities.

Regarding the cold storage facilities of the three ports evaluated (APPENDIX D to F), the lowest counts were recorded for the first European harbour (Fig. 5). Counts for used boxes at European harbour nr. 2 were high. Total average bacterial counts were always greater than for the fungi and yeasts (Fig.5), while it was the contrary for microbial diversities (Fig. 6).

For the reefer ships (APPENDIX G), average microbial counts were higher for the floors at both harbours (Fig.7). Fungal and yeast diversities were higher than for the bacteria, while bacterial dominated the total counts (Fig. 8).

Table 1 Microbial species frequently isolated throughout the fruit chain from South Africa to Europe

Species isolated	Point of isolation	Known significance	Reference
<i>Aeromonas hydrophila</i>	Toilet tap on farm	Occur in fresh water and sewage. Some are pathogenic to frogs, fish and humans. Human diseases are usually diarrhoea or bacteremia	Carter, 1984
<i>Aeromonas salmonicida</i>	Walls and air-con in cold storage room, unused pulp trays, rollers, brushers in packinghouse	Occur in fresh water and sewage. Some are pathogenic to frogs, fish and humans. Human diseases are usually diarrhoea or bacteremia	Carter, 1984
<i>Agrobacterium radiobacter</i>	Fruit pickers' hands on farm	Soil inhabitant., not pathogenic to plants.	Palleroni, 1984
<i>Arcanobacterium bernardiae</i>	Silicon fastener in truck on port	Obligate parasites of the pharynx of humans and farm animals. Members of this genus occasionally cause pharyngeal or skin lesions	Jones and Collins, 1984
<i>Arthrobacter histidinovorans</i>	Walls in storage room of retail centre	Widely distributed in the environment, principally in soil	Jones and Collins, 1984
<i>Arthrobacter ilicis</i>	Brushers in packinghouse on farm	Widely distributed in the environment, principally in soil	Jones and Collins, 1984
<i>Bordetella avium</i>	Picking bags on farm	Members of this genus are mammalian parasites and pathogens	Palleroni, 1984
<i>Brevibacterium casei</i>	Unused boxes on farm	The genus is widely distributed in dairy products and found on the human skin	Jones and Collins, 1984



Table 1 continued

<i>Brevibacterium liquefaciens</i>	Walls in cold room on farm	The genus is widely distributed in dairy products and found on the human skin	Jones and Collins, 1984
<i>Brevundimonas vesicularis</i>	Walls in unwashed container, walls in cold room on harbour	Saprophytic or an opportunistic animal pathogen. Members of the genus are isolated from water and clinical specimens.	Palleroni, 1984; Segers <i>et al.</i> , 1994
<i>Burkholderia cepacia</i>	Floors in cold room of repacking facility	Saprophytic or an opportunistic animal pathogen. Members of the genus are pathogenic to either human and animal or plant	Yabuuchi <i>et al.</i> , 1992
<i>Burkholderia pseudomallei</i>	Floors in cold room of repacking facility	Probably a soil organism and an accidental pathogen, causing melioidosis in animals and humans. Members of the genus are pathogenic to either human and animal or plant.	Yabuuchi <i>et al.</i> , 1992
<i>Cellulomonas flavigena</i>	Walls in unwashed containers on harbour	Widely distributed in soils and decaying vegetable matter	Jones and Collins, 1984
<i>Citrobacter freundii</i>	Floors in truck on harbour	Has been isolated from humans, mammals, birds, reptiles, amphibians, soil and water. Opportunistic pathogen.	http://medical.webends.com/kw/Citrobacter%20freundii
<i>Corynebacterium bovis</i>	Floors in storage room of retail centre	The genus includes primarily obligate parasites of mucous membranes or skin of mammals. Occasionally found in other sources	Jones and Collins, 1984



Table 1 continued

<i>Corynebacterium jeikeium</i>	Fruit packers' hands on farm	The genus includes primarily obligate parasites of mucous membranes or skin of mammals. Occasionally found in other sources	Jones and Collins, 1984
<i>Corynebacterium nitrilophilus</i>	Unused pulp trays on farm	Primarily obligate parasites of mucous membranes or skin of mammals; occasionally found in other sources	Jones and Collins, 1984
<i>Corynebacterium variable</i> (<i>Caseobacter polymorphus</i>)	Unused boxes on farm	The genus includes primarily obligate parasites of mucous membranes or skin of mammals	Jones and Collins, 1984
<i>Enterobacter cloacae</i>	Unused boxes at repacking facility	An opportunistic pathogen, causing burn, wound and urinary tract infections and occasionally septicaemia and meningitis.	Carter, 1984
<i>Klebsiella oxytoca</i>	Customers' hands at retail centre	An opportunistic pathogen that can cause bacteremia, pneumonia and urinary tract and other human infections. It also frequently causes nosocomial infections in urological, neonatal, intensive care and geriatric patients	Carter, 1984
<i>Lactobacillus spp.</i>	Conveyer belt in packinghouse, floors in cold room on farm	Widely distributed in the environment, especially in animal and vegetable food products. Normally inhabit the gastrointestinal tracts of birds and mammals and the mammalian vagina	Kandler and Weiss, 1984



Table 1 continued

<i>Leifsonia aquatica</i>	Walls in cold room on harbour	Genus includes primarily obligate parasites of mucous membranes or skin of mammals. Occasionally found in other sources. Some species are pathogenic for mammals	Jones and Collins, 1984
<i>Microbacterium maritopicum</i>	Rolers in packinghouse on farm	Found in dairy products, sewage and insects	Jones and Collins, 1984
<i>Microbacterium saperdae</i>	Unused boxes at repacking facility, flaps in packinghouse on farm	Found in dairy products, sewage and insects	Jones and Collins, 1984
<i>Micrococcus spp.</i>	Unused boxes on farm, walls in unwashed containers on harbour	Occur primarily on the mammalian skin and in soil, but are commonly isolated from food products and air	Schleifer, 1984
<i>Moraxella spp.</i>	Floors in washed containers on harbour	Parasitic on the mucous membranes of humans and other warm-blooded animals	http://freepages.pavilion.net/tetrix/moraxella.html
<i>Ochrobactrum anthropi</i>	Floors in packinghouse on farm	Occur in human clinical specimens	Bergey <i>et al.</i> , 1994
<i>Pasteurella haemolytica</i>	Walls in cold room on farm	Causes pneumonia and “shipping fever” in cattle, sheep and goats.	Carter, 1984
<i>Pseudomonas stutzeri</i>	Flaps in cold room on harbour	Saprophytic or an opportunistic animal pathogen	Palleroni, 1984

Table 1 continued

<i>Pseudomonas fluorescens</i>	Walls in washed container on harbour, picking bags on farm, floors in cold room on harbour	Saprophytic or an opportunistic animal pathogen	Palleroni, 1984
<i>Ralstonia pickettii</i>	Walls in cold room on harbour	Have been isolated from various clinical specimens and hospital environments with doubtful pathogenicity	Palleroni, 1984
<i>Rhodococcus globerulus</i>	Sizers in packinghouse on farm	This genus is widely distributed, but particularly abundant in soil and herbivore dung. Some strains are pathogenic for animals, including humans	Lechevalier, 1984
<i>Rhodococcus rhodochrous</i>	Walls in cold room on farm	This genus is widely distributed, but particularly abundant in soil and herbivore dung. Some strains are pathogenic for animals, including humans	Lechevalier, 1984
<i>Sanguibacter keddieii</i>	Walls in truck on harbour	Has been isolated from blood of healthy cows	Fernandez-Garayzabal <i>et al.</i> , 1995
<i>Shewanella putrefaciens</i>	Toilet tap on farm	Generally associated with aquatic or marine habitats	Palleroni, 1984

Table 1 continued

<i>Stenotrophomonas maltophilia</i>	Floors, silicon fasteners, air-con in trucks on harbour, silicon fastener on unwashed containers on harbour, fruit picker's hands and wooden pallet base on farm, floors in packing-area and walls in cold room of repacking facility	Members of the genus are isolated from assorted natural materials, human infections and clinical materials	Palleroni and Bradbury, 1993
<i>Tsukamurella inchoensis</i>	Floors in unwashed containers on harbours	Isolated from soil, human sputum and mycetomes and ovaries of bed bugs (<i>Cimex lectularis</i>). Some strains cause infections of the lung, lethal meningitis or necrotizing tenosynovitis	http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=11283073
FUNGI			
<i>Penicillium aurantiogriseum</i> Dierckx	Floors in unwashed containers on harbour, walls in cold storage of repacking facility	Among the most commonly encountered fungi on earth; ubiquitous in many foods; rarely isolated from soils	Pitt, 1988
<i>Penicillium commune</i> Thom	Fruit picking bags on farm	Of common occurrence in foods and other habitats	Pitt, 1988
<i>Penicillium crustosum</i> Thom	Walls of unwashed container on harbour, walls of retail centre, unused pulp trays in packinghouse of farm	Ubiquitous spoilage fungus; weak pathogen of pomaceous fruits; common biodeteriogen	Pitt, 1988

Table 1 continued

<i>Penicillium glabrum</i> (Wehmer) Westling	Walls of unwashed container on harbour, used pulp trays on harbour	Cosmopolitan; widely distributed in soils; biodeteriorative agent	Pitt, 1988
<i>Penicillium glandicola</i> (Oudem.) Seifert & Samson	Silicon fasteners of unwashed container on harbour	Occupies a wide range of habitats, has been isolated rather infrequently	Pitt, 1988
<i>Penicillium janthinellum</i> Biourge	Walls of unwashed container on harbour, fruit packer's hands in packinghouse on farm, walls in cold room of repacking facility, repacker's hands at repacking facility	Ubiquitous soil fungus	Pitt, 1988
<i>Penicillium melini</i> Thom	Silicon fasteners, walls and floors of unwashed containers on harbour, walls of washed containers on harbour	Distribution widespread, though sparse, exclusively a soil fungus	Pitt, 1988
<i>Penicillium spinulosum</i> Thom	Floors of unwashed container on harbour, walls in packinghouse on farm, walls in retail centre	Ubiquitous; commonly occurring in soils, decaying vegetation, foods and textiles; frequently isolated from substrates containing heavy metals, acids and tannins	Pitt, 1988

3.3 REPACKING FACILITIES

Average microbial total counts were the greatest for the floors at repacking facility nr.1 and packers' hands at repacking facility nr.2 (Fig. 9; APPENDIX H and I). The walls at both the facilities yielded the lowest counts when analysed. Microbial diversity was the smallest for the hands of the repacking personnel (Fig. 10).

3.4 RETAIL CENTRES

On average, total microbial counts were the highest for the customers' hands and the lowest for the walls at both retail centres (Fig. 11; APPENDIX J and K). Bacteria dominated counts in all cases. Microbial diversity was the greatest for the hands of the customers that were sampled at retail centre nr.2, and the smallest for the floor surfaces at both centres (Fig. 12).

4. DISCUSSION

In this study it was found that all environments that exported fruit is moved through, exceeded the international hygiene standard for cleaning efficacy of $<5 \text{ cfu/cm}^2$ (Dancer, 2004). Since the standard for food premises is very strict and perhaps unrealistic for fresh fruit handling/holding facilities, future research should include development of a more realistic hygiene standard for fresh fruit environments. This is the first study of its kind where the whole export chain was analysed, and not only facets of the chain. Storage conditions affect both the final populations and types of microorganisms that will grow on or in fresh produce (Madden and Joseph, 1993). The microbial loads of environmental surfaces reflect the efficacy of cleaning programs (Evans *et al.*, 2004) and effective cold chain management (Madden and Joseph, 1993).

Throughout this experiment, it was observed that bacterial counts normally yielded higher counts than fungi and yeasts. Fungi normally require living objects and free water to survive and produce spores, although they also spread with the aid of external factors like air conditioning systems (Hamada and Fujita, 2002). Fungi generally grow better at

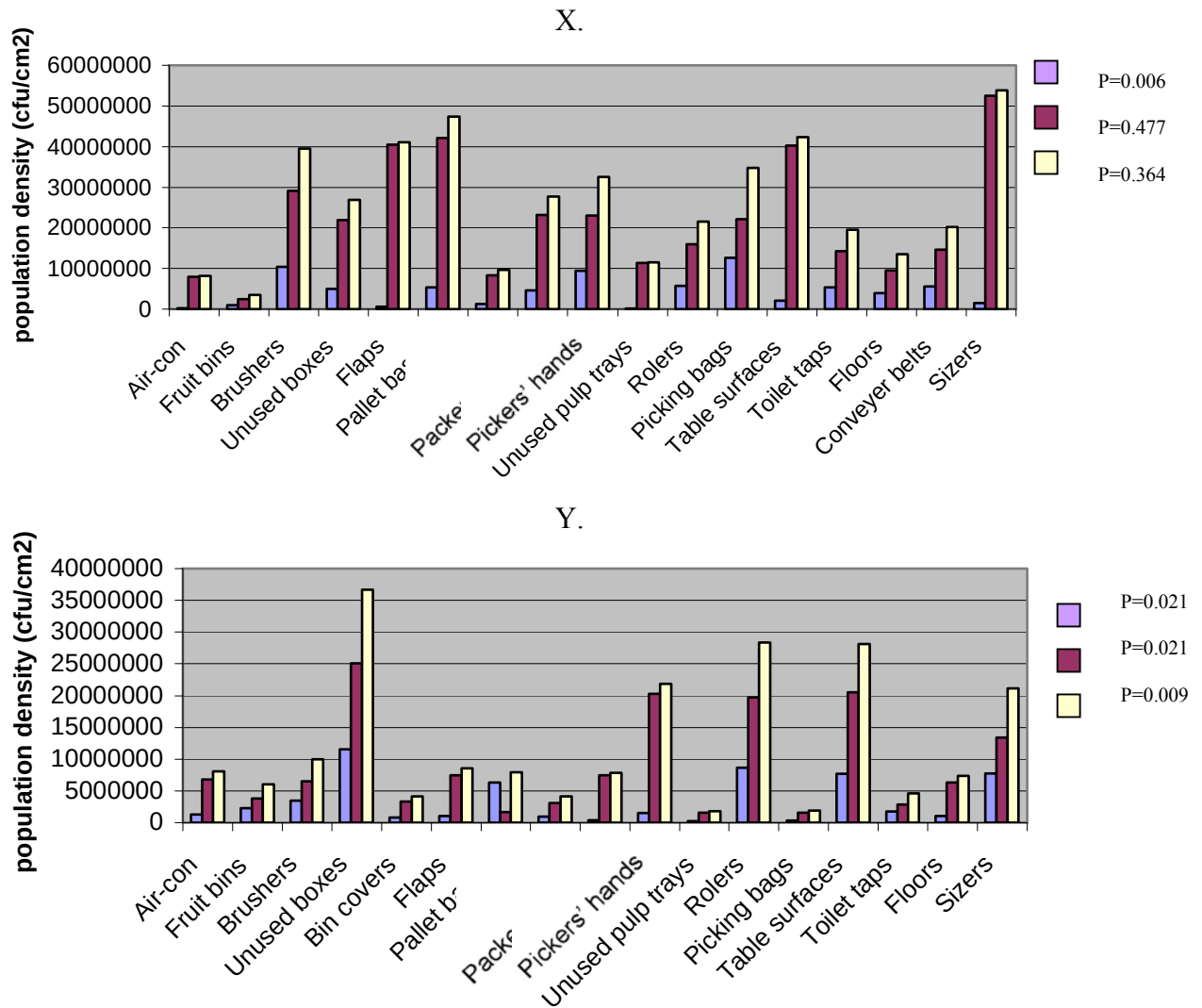


Figure 1 Average microbial total counts of various surfaces sampled at two farms in South Africa.

X= Farm 1

Y= Farm 2

■ Fungal and yeast counts
 ■ Bacterial counts
 ■ Total microbial count

X.

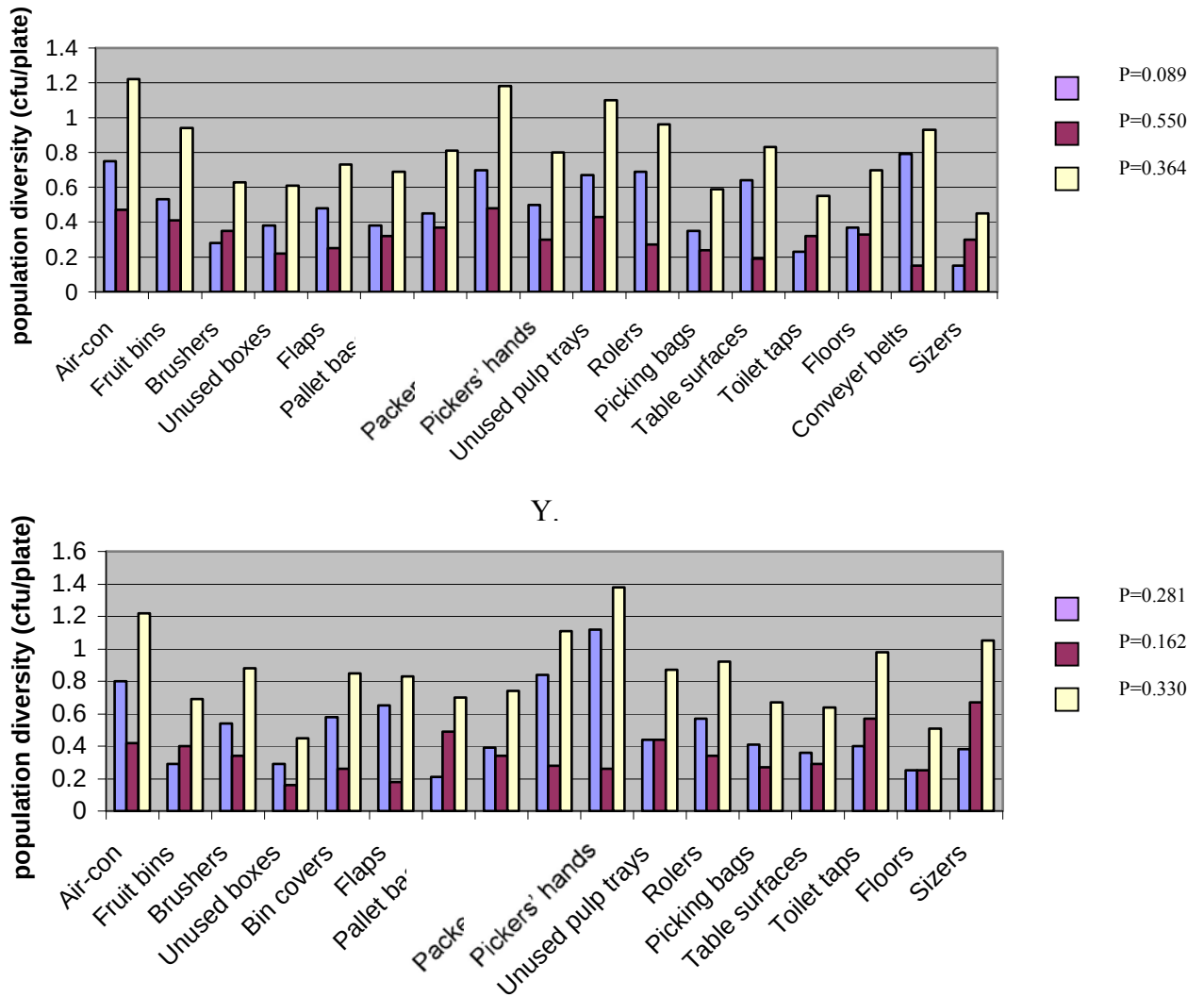


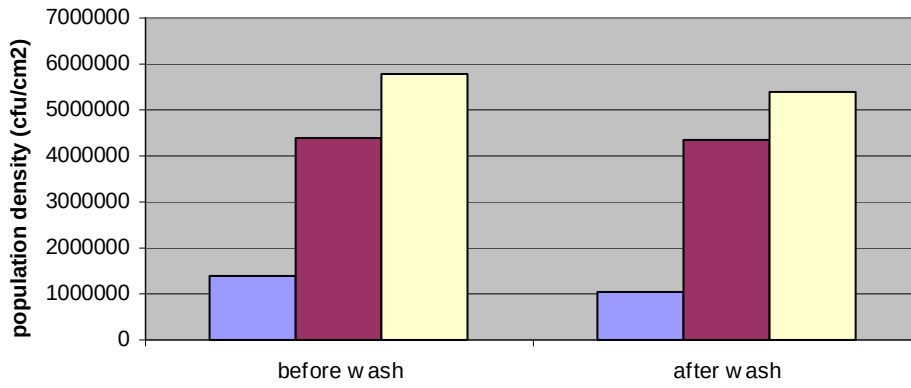
Figure 2 Average microbial diversities of various surfaces sampled at two farms in South Africa.

X= Farm 1

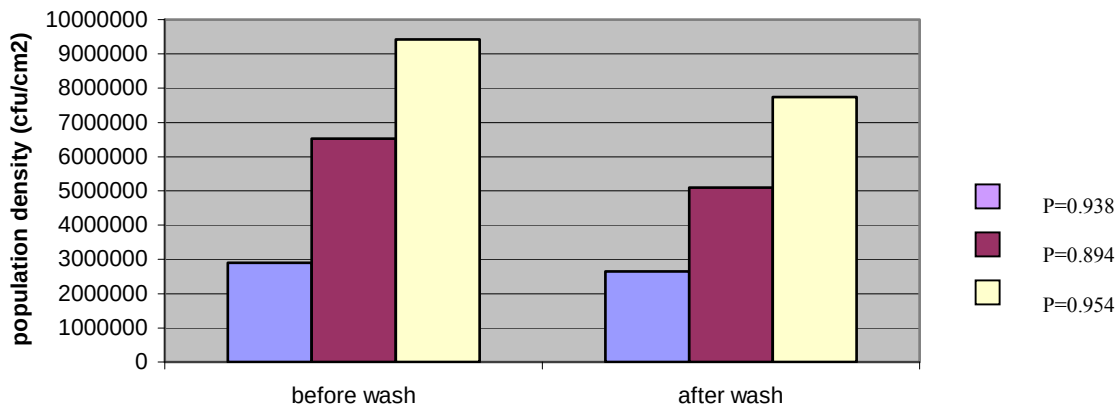
Y= Farm 2

Fungal and yeast diversities
 Bacterial diversities
 Total microbial diversity

X.



Y.



Z.

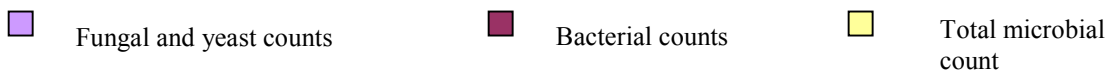


Figure 3 Average microbial total counts of surfaces in unwashed and washed containers on South Africa's harbour.

X= walls

Y= silicon fasteners

Z= floors



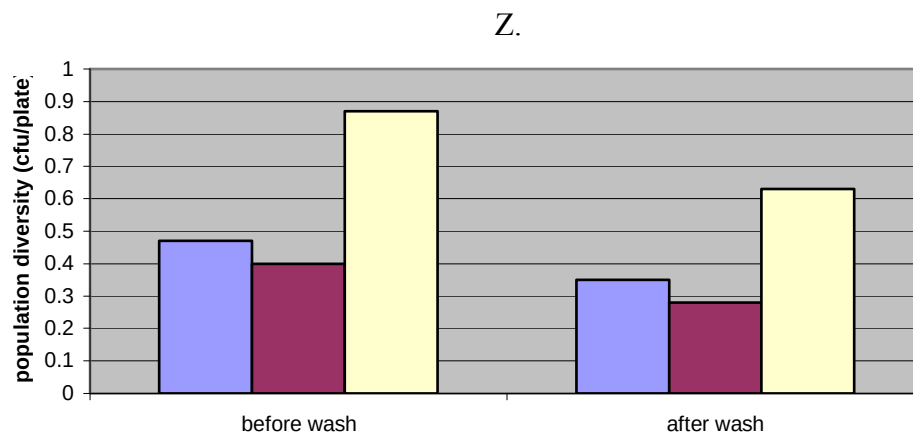
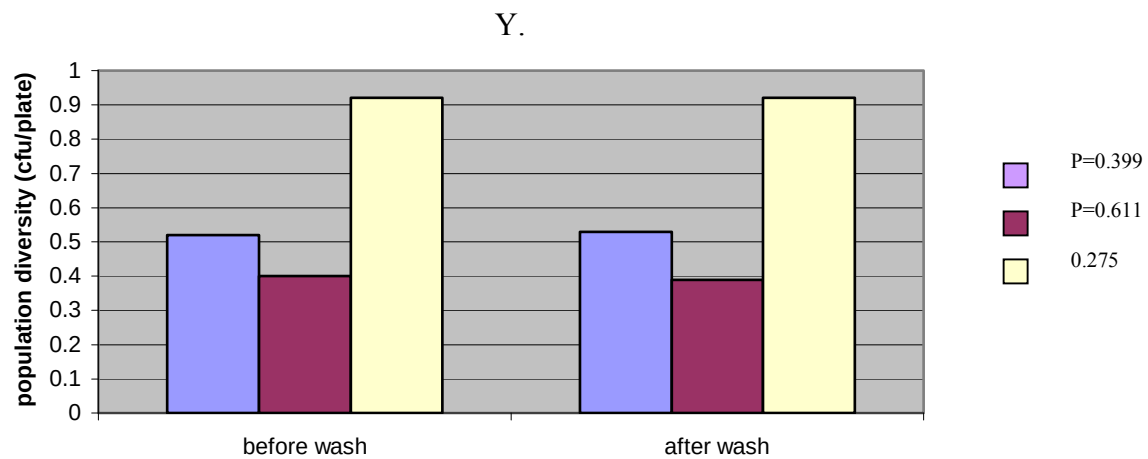
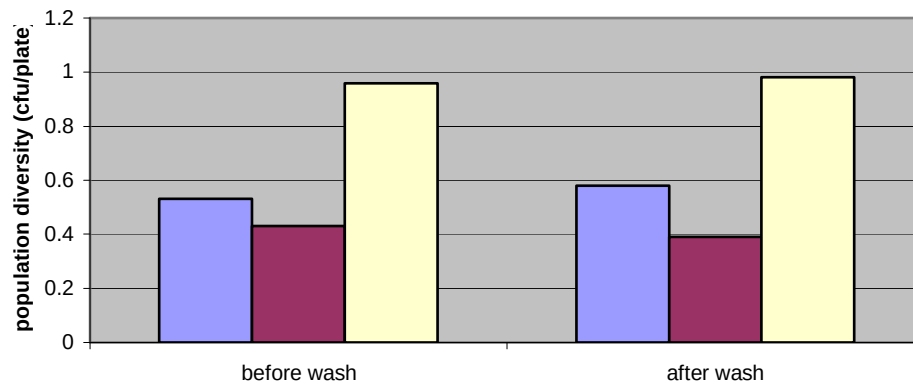


Figure 4 Average microbial diversities of surfaces in unwashed and washed containers on South Africa's harbour.

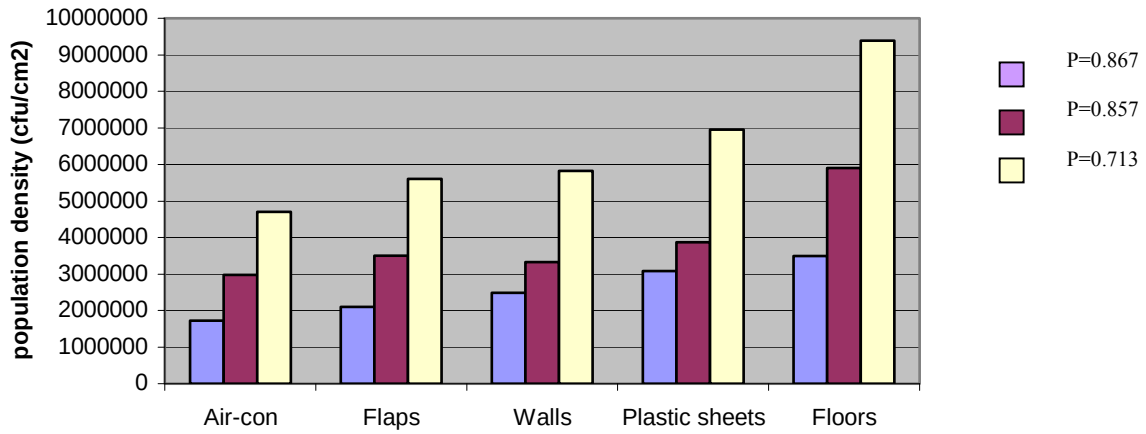
X= walls

Y= silicon fasteners

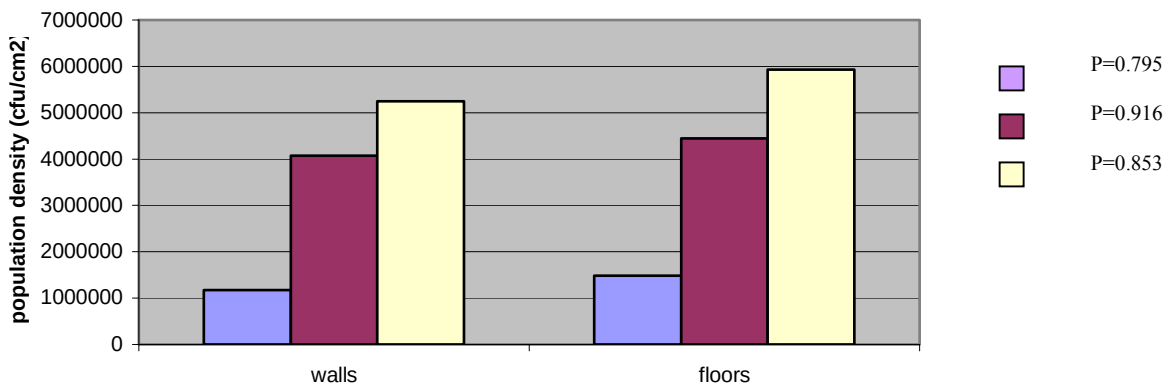
Z= floors

Fungal and yeast diversities
 Bacterial diversities
 Total microbial diversity

X.



Y.



Z.

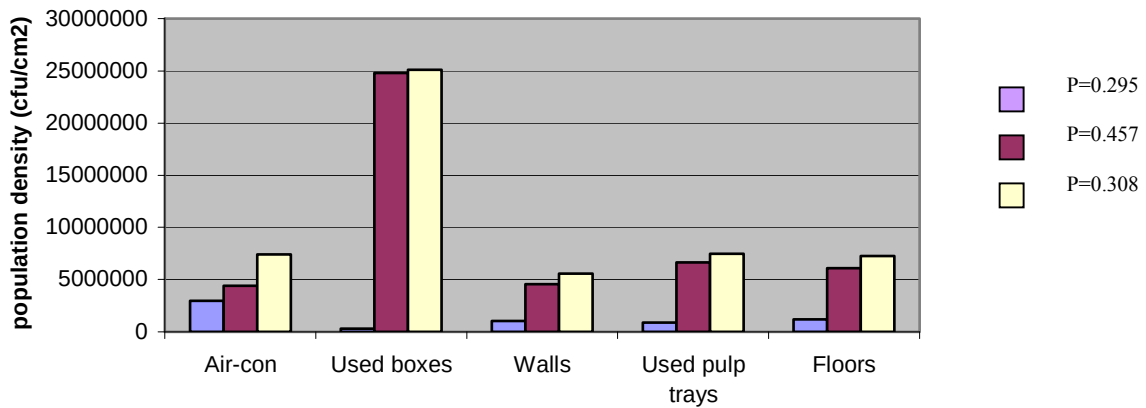
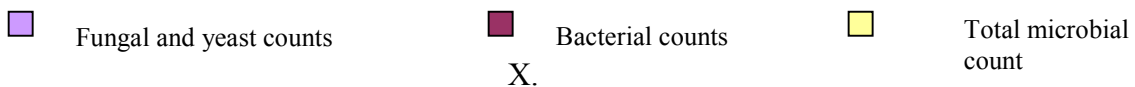


Figure 5 Average microbial total counts for various surfaces in cold storages on three harbours.

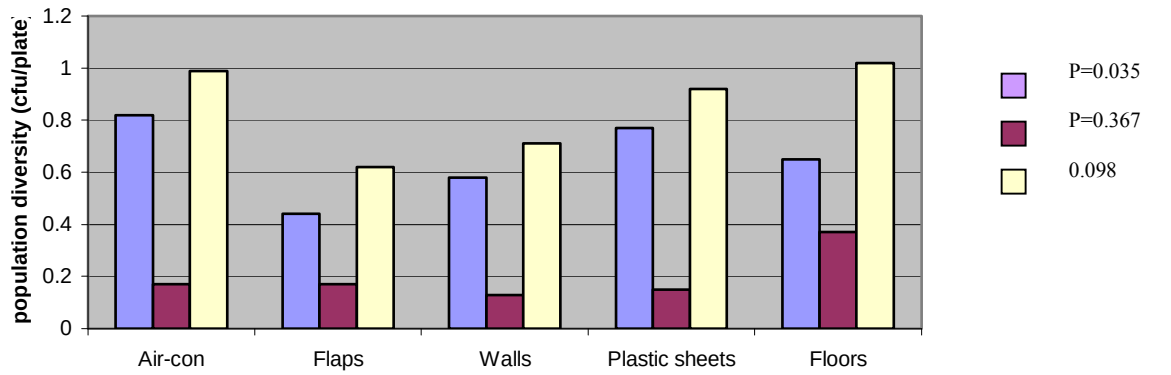
X= South Africa harbour's cold storages

Y= European harbour 1's cold storages

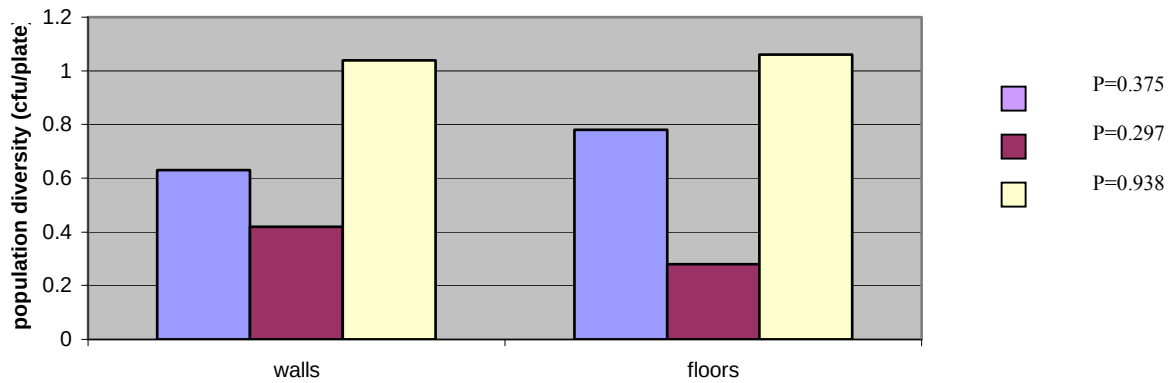
Z= European harbour 2's cold storages



X.



Y.



Z.

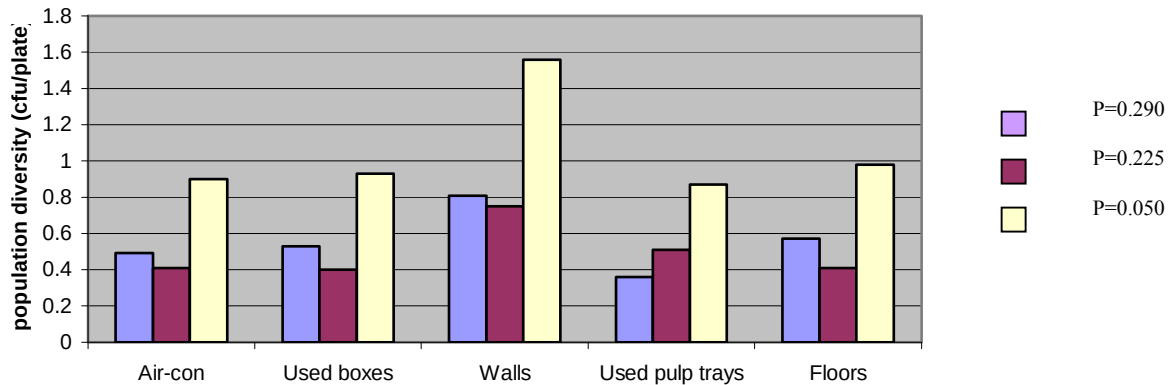
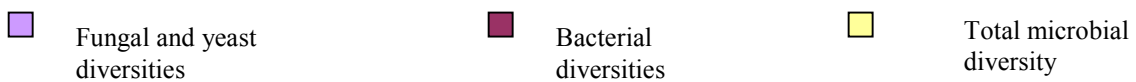


Figure 6 Average microbial diversities for various surfaces in cold storages on three harbours.

X= South Africa harbour's cold storages

Y= European harbour 1's cold storages

Z= European harbour 2's cold storages



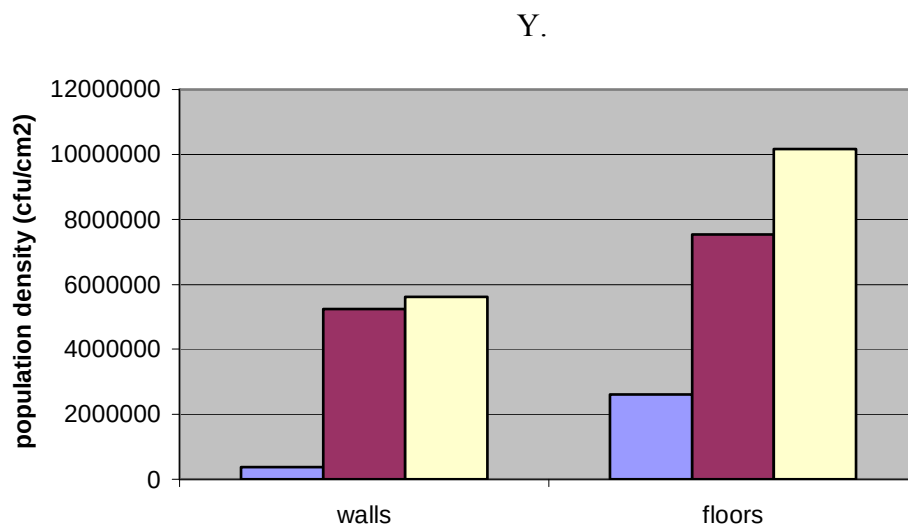
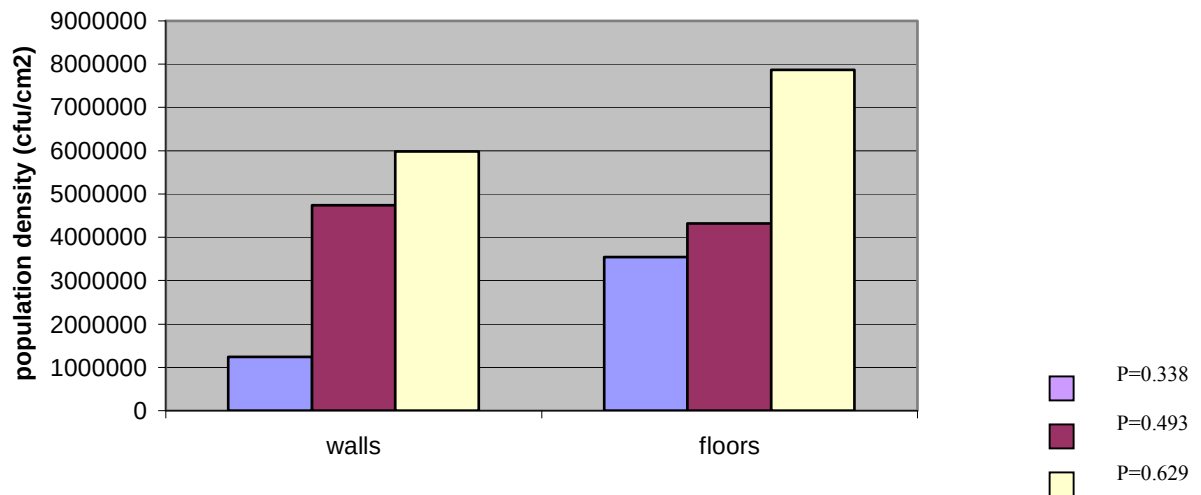
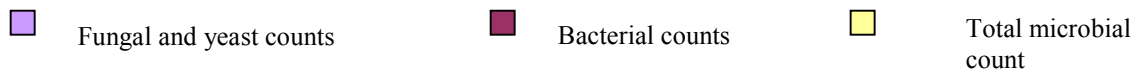


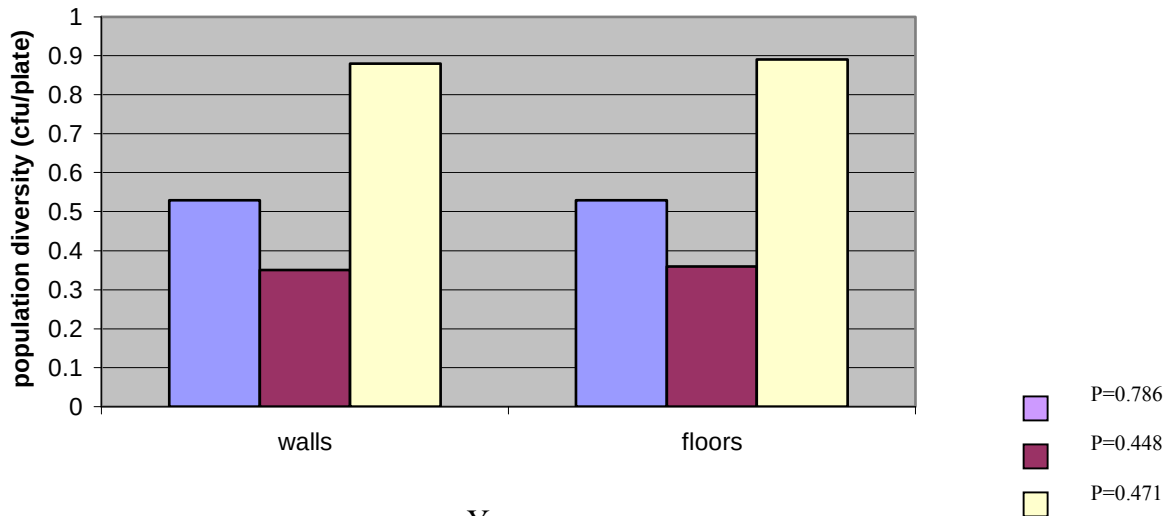
Figure 7 Average microbial total counts of surfaces sampled on ships at two harbours.

X= Ship on South Africa's harbour

Y= Ship on European harbour



X.



Y.

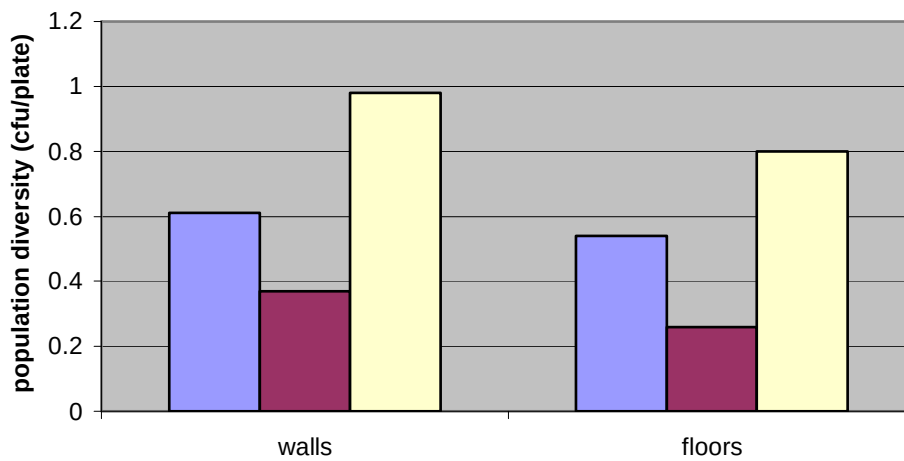
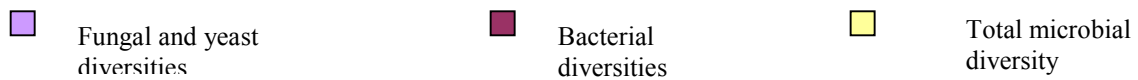


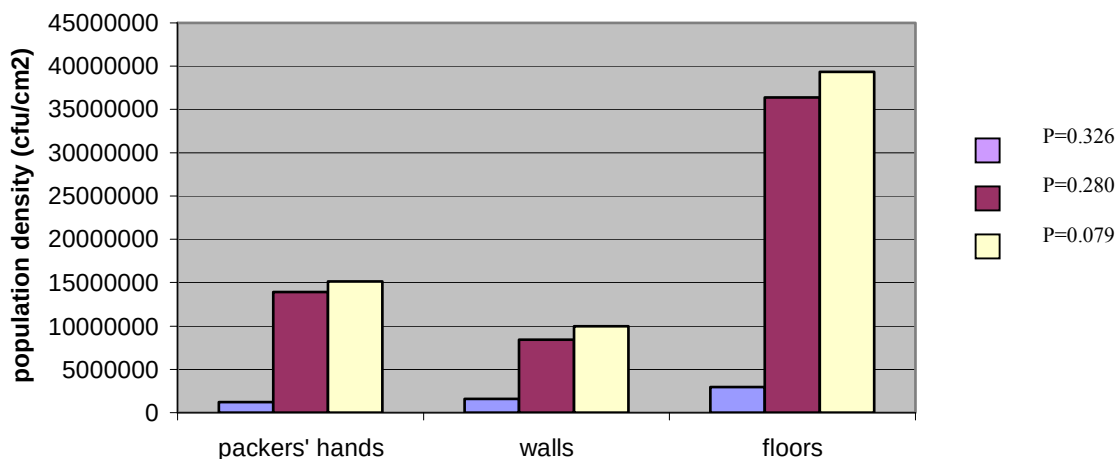
Figure 8 Average microbial diversities of surfaces sampled on ships at two harbours.

X= Ship on South Africa's harbour

Y= Ship on European harbour



X.



Y.

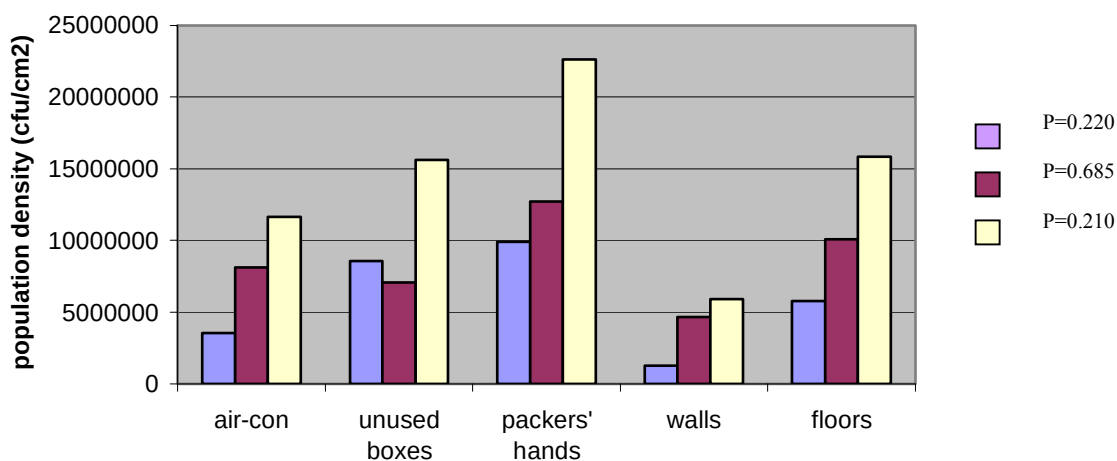


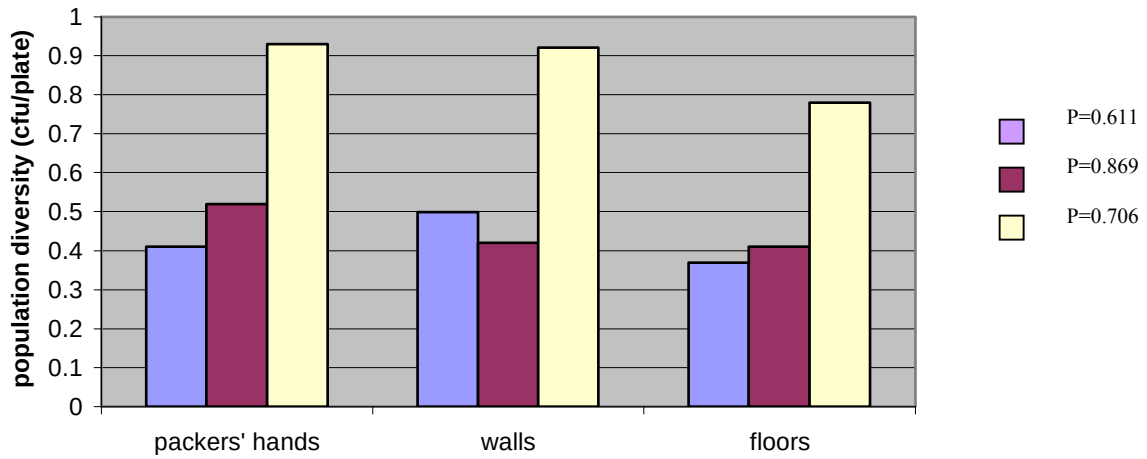
Figure 9 Average microbial total counts of surfaces sampled at two repacking facilities in Europe.

X= Repacking facility 1

Y= Repacking facility 2

■ Fungal and yeast counts
 ■ Bacterial counts
 ■ Total microbial count

X.



Y.

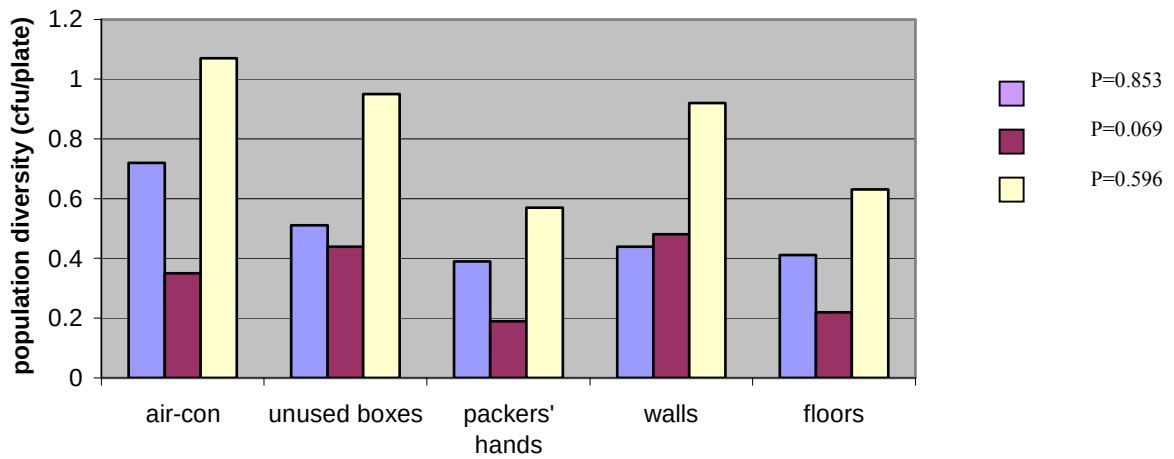


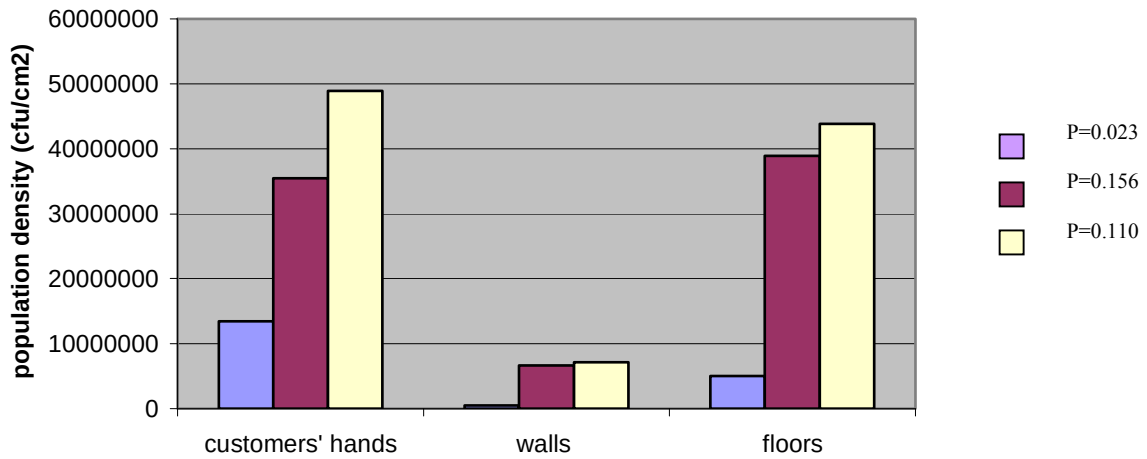
Figure 10 Average microbial diversities of surfaces sampled at two repacking facilities in Europe.

X= Repacking facility 1

Y= Repacking facility 2

■ Fungal and yeast diversities
 ■ Bacterial diversities
 ■ Total microbial diversity

X.



Y.

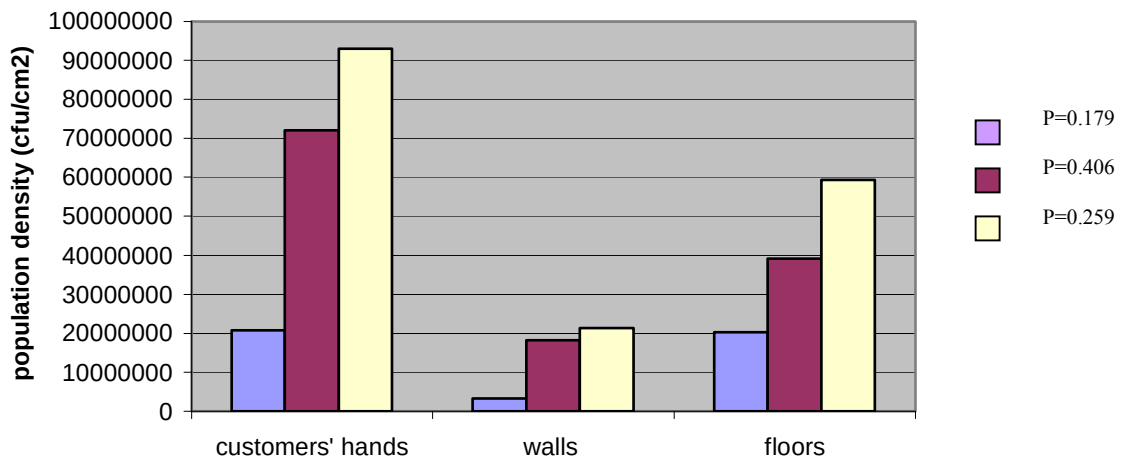


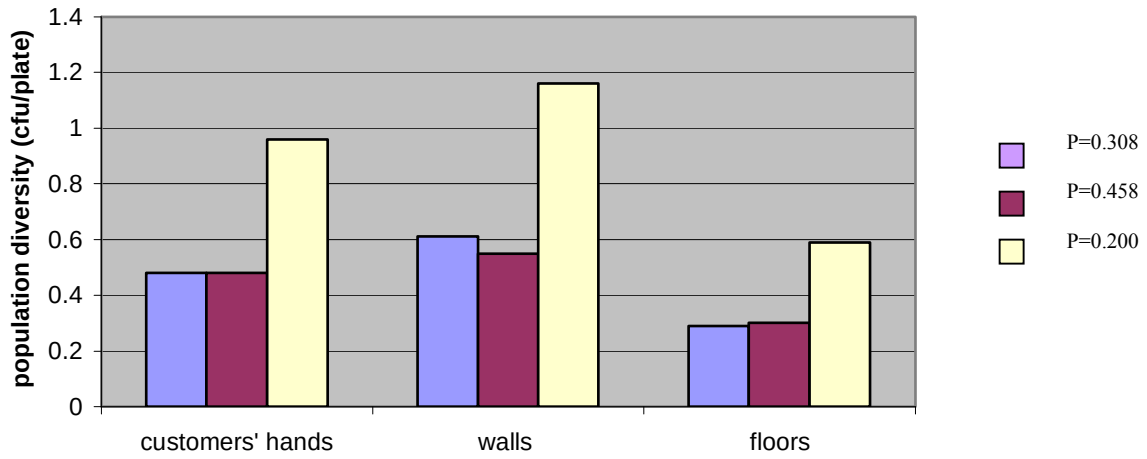
Figure 11 Average microbial total counts of surfaces sampled at two retail centres in Europe.

X= Retail centre 1

Y= Retail centre 2

■ Fungal and yeast counts
 ■ Bacterial counts
 ■ Total microbial count

X.



Y.

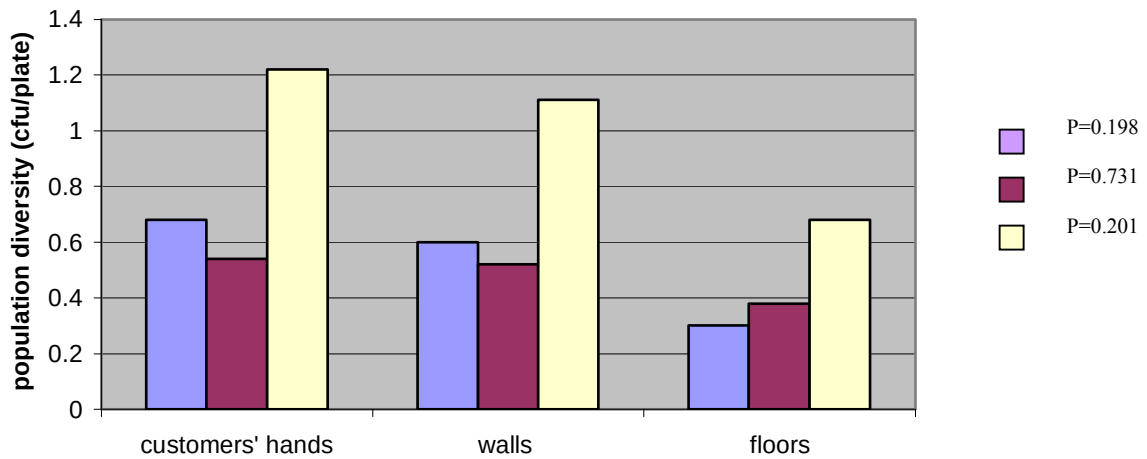
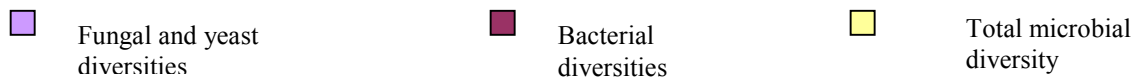


Figure 12 Average microbial diversities of surfaces sampled at two retail centres in Europe.

X= Retail centre 1

Y= Retail centre 2



low pH (Carlile and Watkinson, 1996), i.e. on and in fruit, and are mostly disseminated by air currents that carry them as inert particles over various distances (Agrios, 1997).

The standard deviation was found to be high in most cases of the study. The fact that the experiment was carried out in different environments, which were not always controlled, could be an explanation for this (Atlas and Bartha, 1998). It also proves that the sanitation levels differed between areas, as was visibly evident in this study. Different commodities are handled daily and stored in cold storages, packing areas, containers and trucks, leading to the establishment of variable microbial populations. Surface textures also varied between sampled sites, ranging from brick and concrete to polystyrene, supporting different growth mediums for microorganisms. Several articles have been published on the formation of microbial biofilms on varying surfaces like stone and internal painted walls (Dubosc *et al.*, 2001; Gorbushina and Petersen, 2000; Lopez-Arce and Garcia-Guinea, 2005).

Regarding species diversity, it was evident that sampled surfaces such as pulp trays, empty unused boxes and toilet taps had lower microbial diversities, because they represent natural clean surfaces that don't support microbial growth, unless moisture, debris and dust are added. Under conditions of stress or disturbance, diversity is markedly low, while stable conditions are normally associated with higher species diversity. Species diversity tends to be low in physically controlled ecosystems and tends to be higher in biologically accommodated ecosystems (Atlas and Bartha, 1998). This also explains lower diversities obtained for floor surfaces that are regularly swiped.

At both farms, sampling areas like brushers, sizers and flaps supported high microbial counts, because they are not regularly cleaned, considering their shape and relative position in the building. Unused boxes that were analysed in this study also yielded high microbial counts, possibly because they are stored (as was found in this study) in areas with higher adjacent physical activities such as people moving in and out of facilities that generates dust and other dirt particles. Surprisingly, farm nr.1 that had a new packing house, supported higher average microbial total counts compared to farm nr.2. The reason for this could be that because it was new, an adequate cleaning program had only been

established recently and was not fully implemented yet, while farm nr.2 was in the final implementation phase of a HACCP (Hazard Analysis Critical Control Point) system.

Overall, higher microbial counts could be observed for floors. It is self-explanatory that numerous organisms will grow on floor surfaces, since there is a lot of contact with moving objects, dust, oil and rotten fruit remaining on the surface. While floors are normally swiped regularly, the dirt is not always removed but only distributed by automated brooms. Consequently, microorganisms that travel on dirt particles (Hamada and Fujita, 2002) will only be displaced.

The average microbial total counts for the air-conditioning systems throughout the study, proved to be very high, with average counts ranging from 10^6 - 10^7 cfu/cm². Contamination of air has been shown to influence the shelf life of stored products (Marek *et al.*, 2003) and can be a health hazard to people suffering from allergy problems. Air-conditioning systems produce a lot of moisture and humid conditions within the unit during operation (Hamada and Fujita, 2002) and debris and substrates suitable for microbial growth lead to build up of contamination on air-conditioning coils (Evans *et al.*, 2004). Because air-conditioning systems are normally fixed onto the roof or behind a metal plate in trucks and containers, they are not regularly cleaned (Hamada and Fujita, 2002). In cases where air-conditioning systems are cleaned occasionally, it usually happens when the system requires attention for maintenance work.

At the South African harbour, washing of containers did not have an impact on microbial communities. Silicon fasteners in containers and trucks supported high microbial growth, even when the fasteners were considerably new. Fungal growth was sometimes visible and was not removed by the washing process. Containers are normally cleaned by steam-cleaning the surface with high-pressure steam that reaches 70°C. Only when taints are observed, are the containers additionally cleaned with chemicals. Reused containers should be thoroughly cleaned, kept in good repair, and used only for the transport of uncontaminated food items (Geldreich and Bordner, 1970).

Plastic sheets that cover fruit pallets at the South African harbour also proved to be a matter of concern. They yielded high average total microbial counts and were visibly black as contaminants built up on the surface. Microbial biofilms could develop on such surfaces and might be a potential source of contamination for fruit (Evans *et al.*, 2004).

The high microbial counts present on hands throughout this study is similar to that found by Nel *et al.* (2004). The human body is a reservoir for numerous microorganisms, with hands being the main agents for cross contamination within a fresh-produce handling establishment. Poor personal hygiene practices, such as neglect in washing hands after visiting a bathroom, may result in up to 10^7 pathogens under the fingernails of the food handler in an abattoir (Nel *et al.*, 2004). Although gloves are sometimes worn, lower microbial loads can only be associated with proper glove usage. Training in general hygiene and glove use should consequently be implemented and continuously improved to ensure efficient decrease of bacterial load on hands, particularly in establishments where hand hygiene control can not be performed properly or inexperienced staff are employed (Ayçiçek *et al.*, 2004).

At the retail level, microbial incidence was high overall. At the retail centre nr.1, fruit was stored together with different nonfruit products at room temperatures, while fruit at the second retail centre was held in cold storage (at 5°C; APPENDIX A). Handling at the retail level is perhaps one of the weakest links in the distribution system (Brackett, 1992), because many retailers are notorious in abstaining from proper refrigeration temperatures and good sanitation (Brackett, 1992; Paull, 1999).

Some human pathogens, such as *Aeromonas hydrophila* (Carter, 1984), *Arcanobacterium bernardiae* (Jones and Collins, 1984), *Burkholderia cepacia* and *Burkholderia pseudomallei* (Yabuuchi *et al.*, 1992) (Table 1) were detected on the surfaces that were sampled. If these organisms are able to survive the remainder of the cold chain, they may pose a potential health risk if the product is exposed to this surface. However, the level of risk is considered extremely low since some of these species are widely distributed (Lechevalier, 1984) and most of them are currently not of concern in food safety.

Regarding the representative fungal isolates identified, most of the *Penicillium* spp. that were isolated (Table 1) are ubiquitous, and were commonly encountered throughout the export chain. Although these species occur frequently in nature and are not always plant pathogens, many of them are associated with food spoilage and could therefore be of great importance in maintaining general fruit quality. Of note was the presence of *P. crustosum* (Pitt, 1988), a weak pathogen of pome fruits that could affect apple quality in the export chain.

Risk assessment, reflected in the Hazard Analysis Critical Control Points principle, supports the view that relevant pathogens are widespread, occurring with great variation in time and space. Absence of protection, therefore, in itself constitutes a hazard (Dancer, 2004). Farmers, truck drivers, loading dock workers, distribution and retail workers all share an important role in preventing cross contamination of fruit and maintaining appropriate temperatures (Brackett, 1999). Since the international standard of <5 cfu/cm² for cleaning efficacy is very strict, it is of utmost importance that a more realistic hygiene standard for fresh fruit environments should be developed.

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CHAPTER 4

IMPACT OF THE APPLE EXPORT CHAIN ON FOOD SAFETY

ABSTRACT

Fresh fruit can act as a vehicle for transmitting infectious pathogens, which is a concern in food safety management systems. At the point when fruit reaches the consumer, the level of microbial contamination is likely to reflect the environment to which the produce had been exposed. Safety levels for fruit export environments were determined by sampling various points along the chain, which includes two farms and a harbour in South Africa and two harbours, two repacking facilities and two retail centres in Europe. The presence of six foodborne pathogens i.e. *Shigella sonnei*, *Salmonella muenchen*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* was monitored from 2400 samples collected throughout the chain. Of these, only *S. aureus* and *E. coli* were recorded. *Staphylococcus aureus* was found in containers and at a retail centre in Europe, while *E. coli* was present in large numbers in fruit washing water on a farm in South Africa. Every step from production to consumption plays a significant role in limiting the presence of foodborne pathogens and thereby ensuring the safety of fresh fruit.

1. INTRODUCTION

For the last two decades, human dietary research has shown that a well-balanced diet promotes good health and reduces diseases (De Roever, 1999). Regardless of the benefits related to eating fresh fruit and vegetables, these products can act as vehicles for transmitting infectious pathogens, which is of great importance in food safety management systems (Meng and Doyle, 2002). With the increase in consumption of fresh fruit and vegetables, an increase in the number of foodborne related illnesses associated with contaminated fresh produce has been reported (FDA, 1998). Each year in the USA alone, foodborne diseases cause approximately 76 million illnesses, 325 000 hospitalisations, and 5000 deaths (Meng and Doyle, 2002), with young children, elderly

people and immuno-compromised individuals being the most vulnerable (De Roever, 1999).

Foodborne pathogens do not cause fruit decay, but reside temporarily on fruit as contaminants. Reported pathogens on lettuce, apples, papaya and carrots among others, include *Shigella sonnei*, *Salmonella muenchen*, *Staphylococcus* spp., enterohaemorrhagic *Escherichia coli* 0157:H7 and *Listeria monocytogenes*. These pathogens represent a major public health concern (Beuchat, 2002; Brackett, 1992; Untermann, 1998). Some of these pathogens, like *Staphylococcus aureus*, *E. coli* and *L. monocytogenes*, are airborne (<http://www.iafalwaysfresh.com/listeria.html>; <http://www.americafruit.com/>), enabling them to be disseminated through air currents. *Listeria monocytogenes* can survive on fresh produce stored at refrigeration temperatures (Beuchat, 1995) and populations of *E. coli* and *S. muenchen* have been recovered from fruit stored at 2°C (Kenney and Beuchat, 2002).

Contamination of products can occur at any of the stages during production (pre- and post-harvest) and distribution. Infection sources include soil, faeces (both of human and animal origin), water (for irrigation and cleaning), ice, animals (including insects and birds), handling of products, harvesting and processing equipment (punctured or bruised produce provides entry sites for pathogens), and transportation (Beuchat, 1995; De Roever, 1999; Johannessen *et al.*, 2002). At the point when fruit are marketed, any microbial contamination present is likely to reflect the environment through which the product has been moved (European Commission, 2002).

Although no illnesses have been linked to the consumption of raw apples (Janisiewicz *et al.*, 1999; Kenney and Beuchat, 2002), apple cider and juice have previously been implicated or found responsible for outbreaks of disease caused by *E. coli* 0157:H7 (Dingman, 2000; Janisiewicz *et al.*, 1999) and *Salmonella* spp. (Miller and Kaspar, 1994; Parnell and Harris, 2003). The first reported outbreak of *E. coli* 0157:H7 associated with the consumption of apple cider occurred in 1980 in Canada (Dingman, 2000) and since then, four reported disease outbreaks have occurred in the United States in the last decade, of which one resulted in the death of a 16-month-old girl (Besser *et al.*, 1993).

The main sources of *E. coli* 0157:H7 may be from apples contaminated by irrigation water, fruit fallen on the orchard floor that risks exposure to contaminated animal faeces, exposure of apples to dust from adjacent pastures, and unhygienic handling by farm workers during harvesting and production (Du *et al.*, 2003). Injury to fruit may also facilitate entrance of the pathogen (Dingman, 2000).

The international guideline for the maximum allowed *E. coli* for fresh-cut vegetables and fruit is less than 100 *E. coli*/g, and that for *L. monocytogenes* is less than 10 *L. monocytogenes*/g (Food Science Australia, 2000). The parameter used for *S. aureus* is 100-1000 cfu/g while *Salmonella* spp. should be absent in 25 g of the product (Stekelenburg, 2001).

The aim of this investigation was to determine whether environments to which apples are exposed during the export chain from farm to retail, have the potential to pose any risk to the consumer due to the presence of foodborne pathogens.

2. MATERIALS AND METHODS

2.1 SURFACE SAMPLING

Environmental sampling was performed at two farms and a harbour in South Africa and two harbours, two fruit repacking facilities and two retail stores in Europe.

2.1.1 Farms

Sampling points included five fruit pickers' and five fruit packers' hands, five picking bags, five fruit bins, five bin-liners, five wooden pallet bases, three wall and three floor surfaces in the cold storage areas, six wall and six floor surfaces in the packing house, five empty unused boxes, five pulp trays, four rollers, four brushers, four flaps, four conveyer belt surfaces, four fruit sizers, four table surfaces and five bathroom basin tap surfaces in and around the packing house. Hygiene monitors (Hylab, Israel) for distinguishing *Salmonella*, *Listeria* and *Staphylococcus* spp. were pressed firmly onto the sampling surface (Moore and Griffith, 2002) for ten seconds. Another specimen for the

identification of *E. coli* and *Shigella* spp. was obtained by rubbing an area of 5 cm² with a transport swab (MW & E, UK) (Rampling *et al.*, 2001). Sampling size was based on facility size or surface area, and total volume of work to be done in a specified period. Each sampling point was therefore tested by three hygiene monitors and one swab.

2.1.2 Harbours

Specimens were taken from five containers, three cold storage facilities and five trucks at representative fixed points. These points consisted of floors, walls, but where applicable, silicon fasteners, flaps and plastic sheets were also sampled. At the South African harbour, containers were sampled before and after washing. Sampling was done as described above. At the South African harbour and a European harbour an available reefer ship that had just off loaded its fruit cargo, was also sampled. Three specimens, each consisting of three hygiene monitors and one swab, were taken from floors and walls respectively on three levels in the reefer ship. Sampling at the South African harbour was done twice for all the areas, except for the reefer ship.

2.1.3 Repacking facilities

Samples were taken from three cold rooms and the packing area at each repacking facility, but where necessary, extra sampling points were selected. Extra points included five packers' hands, five empty unused boxes and five pulp trays. In the cold rooms, three replicates were taken from floors and walls, while in the packing areas four replicates were performed as described before.

2.1.4 Retail stores

The holding facility, display area (floors and walls) and five customers' hands were sampled as previously described.

2.2 SURFACE SAMPLE PROCESSING

All samples were transported in cooler boxes and processed promptly. The transport swab of each replicate was inoculated in a test tube with nine ml Brilla broth solution (Biolab, Johannesburg) and incubated for 48h at 37°C. The solution was mixed thoroughly using a vortex shaker (Labotec, South Africa) and 100 microlitres from each test tube was spread plated onto a MacConkey (Biolab) selective media plate. The selective MacConkey plates and hygiene monitors were incubated for 48h at 37°C, after which selected colonies were purified, preserved by freeze-drying (Dhingra and Sinclair, 1985) and identified with the aid of reference cultures and the API system (Biomérieux, France). The reference cultures were obtained from the American Type Culture Collection (ATCC) and included *L. monocytogenes* (#19115), *S. muenchen* (#8388), *S. sonnei* (#9290), *S. aureus* (#12600), *S. epidermidis* (#14990) and *E. coli* 0157:H7 (#35150). Isolates positively identified as one of above-mentioned pathogens were further characterized to test for pathogenicity. Pathogenicity of the isolates was confirmed by performing coagulase, phosphatase, catalase (Holding and Collee, 1971), DNase, and manitol (Jeffries *et al.*, 1957) tests.

2.3 WATER SAMPLING PERFORMED AT THE TWO FARMS

Water sampling was done at the two farms in South Africa. One thousand four hundred millilitres of water was collected from the irrigation source, bathroom tap in the restroom facilities and fruit wash water on both farms. After being transported to the lab in a cooler box within 12 hours, the water samples were analysed immediately. One thousand millilitres was filtered through a 45 µm membrane with a vacuum pump (Balzers, Germany). The membrane was cut into three equal parts and each fraction was transferred to a tube containing a selective enrichment broth. Nine millilitre enrichment broth was prepared from Baird Parker (Biolab), Brilla and Fraser broth (Sharlau, Spain) according to the manufacturer's specifications. After 48h incubation at 37°C, the solutions were mixed thoroughly using a vortex shaker (Labotec, South Africa). Hundred microlitres from each test tube was spread plated onto corresponding selective solid media [Baird Parker (Biolab), MacConkey (Biolab), Listeria (AES Laboratoire, France)].

The selective solid media plates were incubated for 48h at 37°C, after which individual representative typical colonies were isolated, purified on Nutrient Agar (Biolab) and preserved by freeze-drying. All cultures were identified with the API system and the reference cultures were included as positive controls. The remaining 400 millilitre of water was used in two Colilert-18 (IDEXX, USA) tests as specified by the manufacturer to indicate the presence of *E. coli* and coliforms.

3. RESULTS

3.1 SURFACE SAMPLING

Of the 2400 samples that were analysed throughout the export chain, only three areas indicated presence of one of the six foodborne pathogens tested for, namely *S. aureus*. These three areas included a wall and floor specimen taken from two different containers on a European harbour, and a floor specimen taken from a retail store in Europe.

3.2 WATER SAMPLING

Escherichia coli is the only pathogen that was found in the water samples analysed at the two farms in South Africa (Table 1). Pathogenicity was not confirmed due to the complexity of further identification and therefore falls out of the scope of this study.

4. DISCUSSION

This is the first study of its kind; investigating the presence of well-known foodborne pathogens in the apple export chain. Of note is the absence of pathogens such as *S. sonnei*, *S. muenchen* and *L. monocytogenes* (Beuchat, 2002; Brackett, 1992; Untermann, 1998) in environments that export apples are exposed to. *Shigella*, *Salmonella* and *L. monocytogenes* have previously been isolated from surfaces like walls, floor drains (Brackett, 1992), hands (Kusumaningrum *et al.*, 2002) and water (De Roever, 1999) and represent a major health concern (Beuchat, 2002; Brackett, 1992; Untermann, 1998).

Table 1 Detection of *E. coli* in water samples collected at two apple farms in South Africa

Sampling point*	<i>Escherichia coli</i> (MPN) in farm 1's water samples	<i>Escherichia coli</i> (MPN) in farm 2's water samples
Source 1	<1	<1
Source 2	<1	14.5
Flume 1	10.8	190.4
Flume 2	5.2	209.8
After sorting 1	<1	NA**
After sorting 2	2.0	NA
Bathroom 1	<1	5.2
Bathroom 2	<1	10.9

*Source water is water samples that were taken from the irrigation source (borehole water); flume and after sorting water are water samples taken from the wash water in the respective packing houses; bathroom is water samples taken from the basin tap in the bathrooms.

**NA= not applicable

The only foodborne pathogens recorded in this investigation were *S. aureus* and *E. coli*. *Staphylococcus aureus* causes nosocomial infections, particularly in immunocompromised people (Lengeler *et al.*, 1999). Many strains of *S. aureus* can survive desiccation and remain viable on dust particles for many weeks up to months (Rampling *et al.*, 2001). *Staphylococcus aureus* was found in two containers in Europe. Currently the container-shipping industry believes that food safety issues are taken care of provided that the produce has been handled appropriately (Eurofruit, 2002), while the real issue may be the containers themselves. Containers that are used for transport of fruit are often not properly cleaned and sanitized, which can lead to the possible development of surface contamination (Beuchat, 2002). Containers are also used to transport all kinds of products (including high-risk products) since no system is currently running to ensure that only specific containers are used to transport fresh fruits.

Although *S. aureus* is part of the permanent microflora of the human skin (Ayçiçek, *et al.*, 2004), none were found on the hands that were sampled during this study. *Staphylococcus aureus* was however found on floors in the display area of a retail centre in Europe. Temperature is critical at every stage of postharvest handling to prevent pathogen growth (Beuchat and Ryu, 1997). According to Brackett (1992), food handling

at the retail level represents one of the weakest links in the distribution system. Many retailers are known for improper management of refrigeration temperatures and maintaining good sanitation systems. Fruits are often stored together in temporary holding facilities before going on display (Brackett, 1992; Paull, 1999).

Escherichia coli 0157:H7 is a highly resistant pathogen capable of survival in water (Kirby *et al.*, 2003). The South African standard for drinking water is 10 MPN *E.coli* / 100 ml, indicating that the status of the water at one of the farms was above the allowable level, with values up to 209.8 MPN/ 100 ml. Although pathogenicity of the isolated *E. coli* was not confirmed and should be considered to determine its risk potential, water was identified as the source of, among others, the worst outbreak of *E.coli* 0157:H7 in Canada to date (Dingman, 2000). *Escherichia coli* 0157:H7 has raised particular concern due to its survival capabilities, its low infectious dose, its potential for causing severe illness in susceptible populations, its incidence globally and the fact that it occurs naturally in cattle and other animals and is therefore generally found in soil (Kirby *et al.*, 2003).

In the last few years there has been an increase in foodborne illnesses primarily due to complexities of the food system, aging of the population, increased appearance of chronic illnesses and compromised immunity, changing awareness of hygiene and associated risks and changes in microorganisms that have become more virulent and adapted to stresses (<http://postharvest.ucdavis.edu>). Food safety research has thus become an important tool in understanding and preventing foodborne illnesses.

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CHAPTER 5

IMPACT OF THE EXPORT CHAIN ON APPLE SAFETY AND QUALITY

ABSTRACT

It is of utmost importance to evaluate apple microflora throughout the whole export chain in order to determine areas where mismanagement of the raw commodity occurs. Mismanagement may lead to microbial contamination of the final end product. Microbial counts and diversities for apples moving through the export chain were determined by tracking fruit from two different farms in South Africa to retail centres in Europe. Fruit was removed at different points along the export chain and the product quality, safety and microbial composition were evaluated at each sampling point. In this study, it was found that apple microflora changes throughout the export process and the incidence of *Staphylococcus* spp. was recorded on apple surfaces for the first time. Postharvest pathogens are known to cause great economic losses in the apple industry, but proved to be of little significance in this investigation. Everyone participating in the production, postharvest handling and storage of apples should be aware of possible contamination sources from production to consumption. This knowledge will aid in ensuring a safe and quality product.

1. INTRODUCTION

The ultimate purpose of the production, handling and distribution of fresh fruit is to satisfy consumer demands. It is generally established that customer satisfaction is related to product quality (Harker *et al.*, 2003, Shewfelt, 1999), while quality in itself can be viewed as the absence of defects or a degree of excellence (Shewfelt, 1999). Postharvest plant pathogens may cause extensive decay, which affects the quality of economically important fruit crops. Spoilage results in reduced shelf life and considerable economic losses to the fruit industry (Marek *et al.*, 2003), to such a degree that it is estimated that the amount of food lost annually due to postharvest disorders can feed approximately 200 million people (Kelman, 1984 from Combrink *et al.*, 1994). Increased awareness of food

safety has led the consumer to expect fruit to be free from pesticide residues and other contaminants (Cross and Berrie, 1996).

Quality and safety of fresh produce depend on its microbiological flora. Fruit carries a natural non-pathogenic epiphytic microflora with the bulk of bacteria being Gram-negative and belonging either to the *Pseudomonas*-group or to the *Enterobacteriaceae* (European Commission, 2002). These microorganisms normally subsist on traces of protein, carbohydrates and inorganic salts which dissolve in the water that exudes from the epidermis of the plant or condenses from the atmosphere onto the plant surface (Madden, 1992). During growth, harvest, transportation and further handling, the produce can become contaminated with pathogens from various sources (De Roever, 1999; European Commission, 2002). Improper handling and unsanitary equipment can, therefore, lead to increased microbial populations and can compromise quality and safety (Spadaro and Gullino, 2004). Storage conditions affect both the final population and types of microorganisms that will grow on or in fresh produce (Madden and Joseph, 1993).

Temperature and relative humidity are possibly the two most essential factors influencing the effect of storage on microflora. Unsuccessful cold chain management contributes to microbial survival and contamination of produce that leads to rejection of export fruit due to postharvest decay (Madden and Joseph, 1993). Fruit is often exposed to non-optimal temperatures during packing, ship loading, distribution to retailers, and in retail outlets (Johnston *et al.*, 2001). The length of time fresh produce is held before consumption can also influence microbial populations (Brackett, 1992; Madden, 1992).

The domestic apple, *Malus X domestica* Borkh., is believed to originate from *M.sieverssii* (Ledeb) M.Roem., a diverse species from the mountains of central Asia (Jones and Aldwinckle, 1990) and is one of the oldest world fruit crops. Apples are by far among the most important deciduous tree fruits and are widely cultivated both in temperate and tropical regions. The fruit is primarily grown for the fresh fruit market and is marketed by cultivar name to a far greater extent than has been for other fresh fruits and vegetables. Effective and rapid long-distance transportation and improved storage conditions have

enabled apple industries to figure prominently in world trade (Jackson, 2003). In the southern hemisphere, South Africa's production increased 12-fold over the period 1948/1950 to 1996 to support market demand (Dall, 1999; Jackson, 2003), so that 80% of its apple production is exported to Europe (Groenewald, 2000). The cultivars produced for export in South Africa include Royal Gala, Pink Lady, Fuji, Big Chief, Top Red, Sundowner, Braeburn, Granny Smith and Golden Delicious (Jooste, 2000).

Fruit has a high moisture content and percentage of carbohydrates and a low pH. For apples, the pH ranges from 2.9 to 5.0 (related to climatic conditions and the length of the growing season), and it is this feature that limits the growth of a broad variety of microorganisms, so that only yeasts, moulds and acidophilic bacteria are capable of abundant growth (Doores, 1983; Downing, 1989). Microorganisms are assumed to be limited to the surface of whole, healthy fruits, whereas it is generally accepted that the internal tissue of the fruit is sterile. Total yeast counts range from 10^2 to 10^6 organisms per apple while individually (Ayres *et al.*, 1980 from Doores, 1983) healthy apples carry a fungal load of 10^3 to 10^5 organisms. Fruit having higher counts is normally infected or damaged (Vismer *et al.*, 1996).

Fungal pathogens are the main cause of postharvest losses of apples that can be as high as 50% of the final product at the retail end (Spadaro *et al.*, 2004). The ultimate cause of many problems in the apple industry may be traced to mismanagement of the raw commodity since the harvesting; handling and storing of apples significantly influence their quality and yield (Downing, 1989). Although no foodborne illnesses have been linked to the consumption of raw apples (Janisiewicz *et al.*, 1999; Kenney and Beuchat, 2002), apple cider and juice have been strongly implicated or found responsible for outbreaks of disease caused by *Escherichia coli* 0157:H7 (Dingman, 2000; Janisiewicz *et al.*, 1999) and *Salmonella* (Miller and Kaspar, 1994; Parnell and Harris, 2003).

The aim of this study was to identify the areas along the export chain that may affect the postharvest safety and quality of export apples.

2. MATERIALS AND METHODS

Royal Gala apples were collected from two orchards on two farms, and fruit from the same batch was followed as it moved through the export chain i.e. sampled at the harbour in South Africa, and in Europe from a harbour, two repacking facilities and two retail stores in Europe.

2.1 COLLECTION OF FRUIT

2.1.1 Farms

At both farms, apples were aseptically picked from five trees in the same orchard to represent untouched fruit. These fruits represented the starting microflora of the fruit chain to which the rest of the fruit in the chain was compared. Apples freshly harvested by pickers from the same orchard row were then taken out of five collecting bins, representing handled fruit. After these bins were connected to a tractor, they were covered and transported on a gravel road (± 2 km) to the packing house. At arrival at the packing house, fruit samples were collected from the five bins representing transported apples. The remaining fruit collected from the original orchard rows was put in a cold room to remove field heat, and after storage overnight, the apples were handled through the packing line. Fruit was washed in untreated water, transported on rollers and conveyer belts, treated with brushers and conveyed to packing personnel who packed the apples into boxes, palletized them, ready for export. From five of these boxes, fruit samples were removed. Each sample consisted of eight apples intended for postharvest pathological studies and eight apples for determining fruit microflora.

To represent the export chain from South Africa to Europe, two pallets from each farm were marked with easy identifiable stickers, coded and tracked from the farm to the port in Europe (Figure 1). Temperature loggers (Sensitech, Dole) were wired to the central box of each pallet to monitor temperature and relative humidity throughout the voyage.

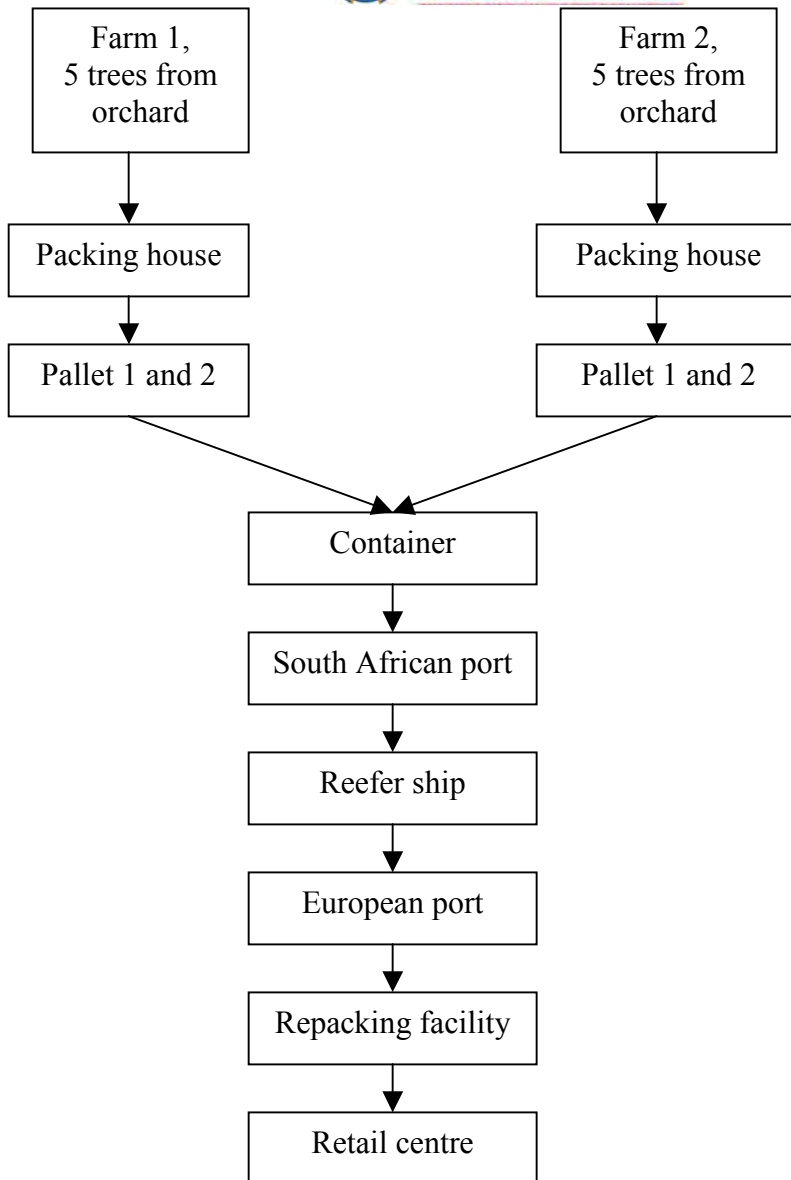


Figure 1 Fruit export chain from South Africa to Europe.

2.1.2 Harbours

In South Africa, the fruit pallets from the farms were transported by cold truck to the harbour (a journey of approximately 16 hours), where the fruit was stored for three weeks before being loaded on a reefer. At this point one of the pallets from farm nr.2 was loaded into the wrong container, unfortunately destined for China. From the remaining pallets, five boxes were removed randomly for apple sampling from the second row of each pallet. The top row was excluded and used as a buffer zone. The space created by

the removed boxes in the pallet was filled with empty boxes. Again, two specimens of eight fruits, representing one fruit sample, were collected per box, as described previously. The three pallets from the two farms were shipped in one integral container intended for export to Europe. After a voyage of 16 days on a reefer ship, the container arrived at a port in Europe. At this point, five boxes were removed from the next two rows and two specimens of eight fruits removed per box as described earlier. Fifteen boxes from each pallet were left at the harbour and inspected by port personnel for postharvest quality. Five boxes from each pallet were transported by road in a car (an hour journey) to two repacking facilities to simulate distribution (cold trucks could not be used due to technical considerations).

2.1.3 Repacking facilities

Five boxes from two of the three pallets, from two farms, were taken to one repacking facility. Five boxes from the remaining second pallet from the one farm were taken to another nearby repacking facility. At each repacking facility, packing personnel were asked to repack the five boxes of fruit into boxes intended for the retail market. The author collected five fruit samples from the handled fruit as described in Section 2.1.1. The remaining handled fruits were transported in their boxes by car the next morning to two retail stores, approximately 2 hours away.

2.1.4 Retail centres

Five boxes from two of the three pallets, from two farms, were taken to one retail store in Europe. Five boxes from the remaining second pallet from the one farm were taken to another retail store in Europe. The fruit of the five boxes from each farm were mixed and displayed in the fruit and vegetable section of the stores. Five customers were asked to handle fruit as if they intended buying them. Three apple samples, consisting of eight fruits each, were collected from the handled fruit.

2.2 FRUIT PROCESSING

The eight fruits from each fruit sample that was intended for microflora investigation were handled in the following manner: apples were washed in an ultrasonic bath (UMC 5, South Africa) with 500 ml Ringer's (Merck, Germany) solution to remove surface microflora. The Ringer's solution was filtered through a 45 µm membrane using a vacuum pump (Balzers, Germany), after which the membrane was cut into four equal parts. Three fractions were transferred to three tubes containing different selective enrichment broths. Nine ml enrichment broths were prepared from Baird Parker (Biolab, Johannesburg), Brilla (Biolab) and Fraser broth (Sharlau, Spain) according to the manufacturer's specifications. After a 48h incubation period at 37°C, the solutions were mixed thoroughly using a vortex shaker (Labotec, South Africa) and 100 µl from each test tube was spread plated onto corresponding selective solid media [Baird Parker (Biolab), Macconkey (Biolab), Listeria (AES Laboratoire, France)]. The selective solid media plates were incubated for 48h at 37°C, after which selected colonies were purified, preserved and identified with the aid of reference cultures and the API Staph, API 20NE and API 20E systems (Biomérieux, France). The reference cultures were obtained from the American Type Culture Collection (ATCC) and those used were *Listeria monocytogenes* (#19115), *Salmonella muenchen* (#8388), *Shigella sonnei* (#9290), *Staphylococcus aureus* (#12600), *S. epidermidis* (#14990) and *E. coli* 0157:H7 (#35150). Isolates positively identified on the selective media according to described typical colony colour and morphology as one of above-mentioned pathogens were further characterized to confirm if they represented a pathogenic culture.

The fourth fraction of the membrane was transferred to a tube containing nine ml Ringer's solution. The solutions were mixed thoroughly using a vortex shaker (Labotec, South Africa) and 100 µl from each test tube was spread plated onto Standard 1 Nutrient Agar (STD1) (Biolab) and Malt Extract Agar (MEA) (Biolab) plates. Three dilutions were performed i.e. 10^{-3} , 10^{-4} and 10^{-5} . The STD1 plates were incubated for 48h and MEA plates for 96 h at 25°C and total counts and species diversity were recorded as described before (Chapter 3, Section 2.2).

The eight fruits from each fruit sample that was intended for postharvest pathological investigations were handled in the following matter: Of the five fruit samples, two samples were wounded to facilitate pathogen entry. The wounds were inflicted by pressing sterile needles, 10 mm apart from each other, five mm deep into the equatorial periphery. The five fruit samples from each batch were left in a room at ambient temperature (25°C, 65% RH) and visually assessed every two weeks up to six weeks after initial sample collection. From every apple that had decay symptoms, isolations were made and grown on Malt Extract Agar (MEA) (Biolab). Identification of the fungal cultures was done by R. Jacobs (Plant Pathology laboratories, Mycological Unit, University of Pretoria).

2.3 ENVIRONMENTAL MONITORING

Temperature was measured throughout the experiment as described in Chapter 3, Section 2.3.

2.4 STATISTICAL ANALYSIS

Statistical analysis was performed as outlined in Chapter 3, Section 2.4.

The aim of the statistical procedure was to identify particular trends in microbial counts and diversities throughout the export chain, and not to determine whether significant differences were present (standard deviations and significance levels are shown in the Appendix and not indicated on graphs).

3. RESULTS

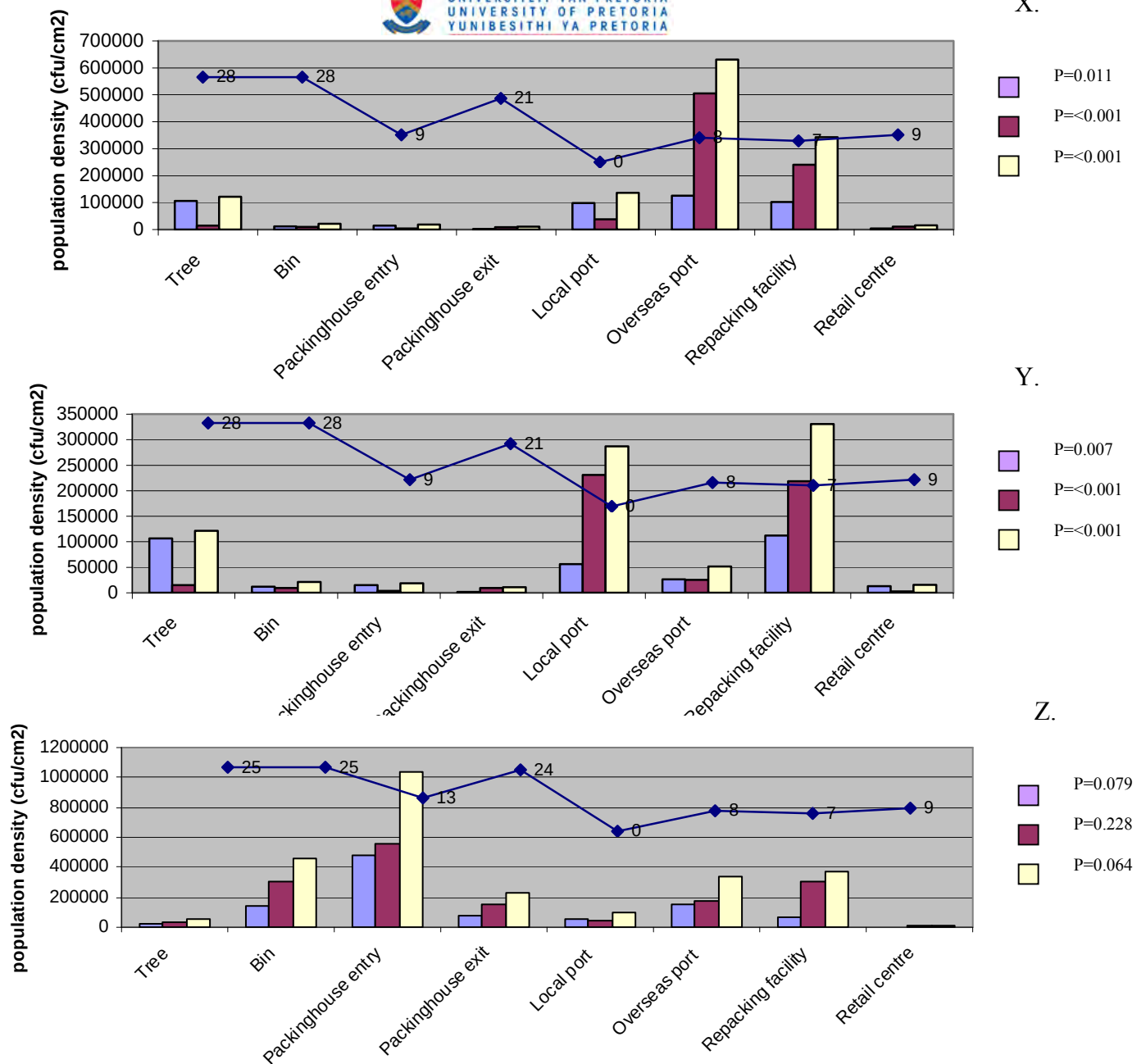
3.1 AVERAGE MICROBIAL TOTAL COUNTS AND DIVERSITIES

Average microbial counts were the greatest for the apples that entered the packinghouse of farm nr.2 (Fig. 2). For pallets X and Y from farm nr.1, there was a decrease in microbial counts from apples that were on the tree to those collected in fruit bins, and an increase in microbial total counts from the farms to the local port. In two of the three

pallets (X from farm nr.1 and Z from farm nr.2) there was an increase in average microbial counts from the local to the overseas port. For all three pallets a decrease in microbial numbers from packinghouse entry to exit and from the repacking facilities to the retail centres was observed (Fig. 2; APPENDIX P to R).

Bacterial diversities were continuously greater for two of the three pallets (X and Y from farm nr.1), except at the packinghouse entry for both cases and at the retail centre for the second pallet (Y), where fungal and yeast diversities were higher. Microbial diversity was the largest for fruit collected at the retail centre for pallet 1 (X from farm nr.1) and at the local port in South Africa for pallet 3 (Z from farm nr.2). The smallest diversities were recorded for apples obtained from the fruit bins of farm nr.2 (Fig. 3; APPENDIX P to R).

Results for the postharvest pathological investigations are given in Tables 1 and 2, while the results for the foodborne pathogen analysis is given in Table 3.



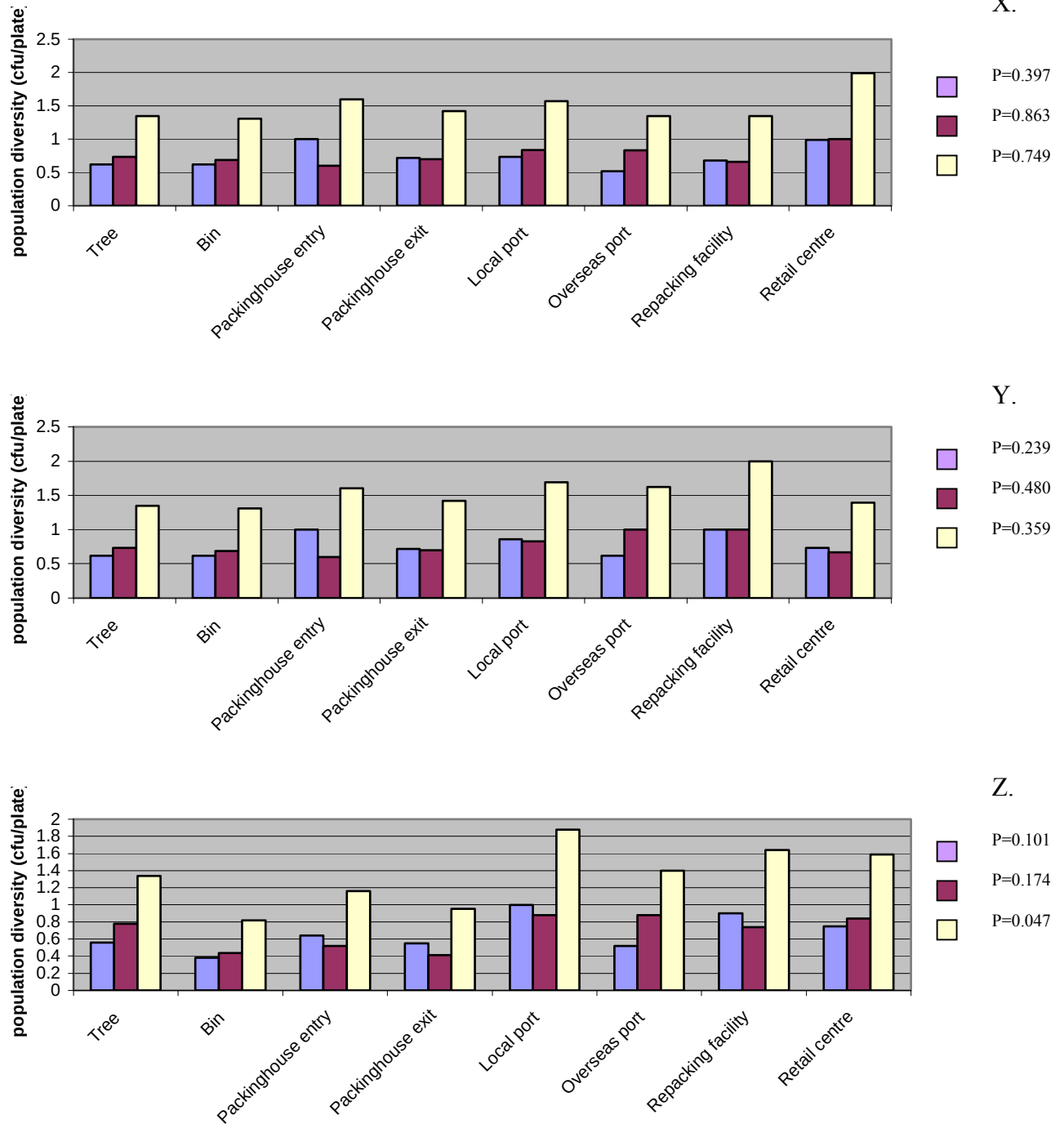


Figure 3 Average microbial diversities on apples of farm 1's two pallets (X and Y) and farm 2's pallet (Z) from the farm in South Africa to a retail centre in Europe.

Fungal and yeast diversities
 Bacterial diversities
 Total microbial diversity

Table 1 Decay analysis of apples distributed from farm 1, evaluated on three different dates (every two weeks for six weeks)

CODE*	Date 1	Date 2	Date 3
Pre-orchard 1 (W)**	1 decay	1 decay	-
Pre-orchard 2 (W)**	-	-	-
Pre-orchard 3	-	-	-
Pre-orchard 4	-	-	-
Pre-orchard 5	-	-	-
Post-orchard 1 (W)**	-	-	-
Post-orchard 2 (W)**	-	-	-
Post-orchard 3	-	-	-
Post-orchard 4	-	-	-
Post-orchard 5	-	-	-
Pack-in 1 (W)**	-	-	-
Pack-in 2 (W)**	-	-	-
Pack-in 3	-	-	1 decay
Pack-in 4	-	-	-
Pack-in 5	-	-	-
Pack-out 1 (W)**	-	-	-
Pack-out 2 (W)**	-	-	-
Pack-out 3	-	-	-
Pack-out 4	-	-	-
Pack-out 5	-	-	-
Local port 1.1 (W)**	-	-	-
Local port 1.2 (W)**	-	-	-
Local port 1.3	-	-	-
Local port 1.4	-	1 decay	1 decay
Local port 1.5	-	-	-
Local port 2.1 (W)**	1 decay	-	-
Local port 2.2 (W)**	-	-	-
Local port 2.3	-	-	-
Local port 2.4	-	-	-
Local port 2.5	-	-	-
Repack 1.1 (W)**	-	-	-
Repack 1.2 (W)**	-	-	-
Repack 1.3	-	-	1 decay
Repack 1.4	-	-	-
Repack 1.5	-	1 decay	-
Repack 2.1 (W)	-	-	-
Repack 2.2 (W)	-	-	-
Repack 2.3	-	-	-
Repack 2.4	-	-	-
Repack 2.5	-	-	-

*The first number indicates the sample number or represents the pallet number, while the second number indicates the box from which the sample was taken.

Pre-orchard= fruit samples taken from trees in the orchard

Post-orchard= fruit samples taken from bins in the orchard

Pack-in= fruit samples taken from bins arriving at the packinghouse

Pack-out= fruit samples taken from boxes ready for export

** (W) indicates wounds that were inflicted to facilitate pathogen entry. Five samples consisting of eight fruits each, were analysed.

Table 2 Decay analyses of apples distributed from farm 2, evaluated on three different dates (every two weeks for six weeks)

CODE*	Date 1	Date 2	Date 3
Pre-orchard 1 (W)**	-	-	-
Pre-orchard 2 (W)**	-	-	-
Pre-orchard 3	-	-	-
Pre-orchard 4	-	-	-
Pre-orchard 5	-	-	-
Post-orchard 1 (W)**	-	-	-
Post-orchard 2 (W)**	-	-	-
Post-orchard 3	-	-	-
Post-orchard 4	-	-	-
Post-orchard 5	-	-	-
Pack-in 1 (W)**	-	-	1 decay
Pack-in 2 (W)**	-	-	-
Pack-in 3	-	-	-
Pack-in 4	-	-	-
Pack-in 5	-	-	-
Pack-out 1 (W)**	-	-	-
Pack-out 2 (W)**	-	-	-
Pack-out 3	-	-	-
Pack-out 4	-	3 decay	-
Pack-out 5	-	-	-
Local port pallet 1.1 (W)**	-	-	-
Local port pallet 1.2 (W)**	-	-	-
Local port pallet 1.3	-	-	-
Local port pallet 1.4	-	-	-
Local port pallet 1.5	-	-	-
Repack 1.1 (W)**	-	-	-
Repack 1.2 (W)**	-	-	-
Repack 1.3	-	-	2 decay
Repack 1.4	-	-	-
Repack 1.5	-	-	-

*The first number indicates the sample number or represents the pallet number, while the second number indicates the box from which the sample was taken.

Pre-orchard= fruit samples taken from trees in the orchard

Post-orchard= fruit samples taken from bins in the orchard

Pack-in= fruit samples taken from bins arriving at the packinghouse

Pack-out= fruit samples taken from boxes ready for export

** (W) indicates wounds that were inflicted to facilitate pathogen entry. Five samples consisting of eight fruits each, were analysed.

3.2 DETECTION OF FOODBORNE PATHOGENS

Table 3 Foodborne pathogens isolated from apples throughout the export chain from South Africa to Europe

Species isolated	Point of isolation	Sample from which isolated
<i>Staphylococcus aureus</i>	South African harbour	Farm 2's pallet, box 1
<i>S. aureus</i>	South African harbour	Farm 2's pallet, box 2
<i>S. aureus</i>	South African harbour	Farm 2's pallet, box 4
<i>S. aureus</i>	South African harbour	Farm 1's pallet 1, box 4
<i>S. epidermidis</i>	European harbour	Farm 1's pallet 2, box 2

4. DISCUSSION

In this study it was indicated that apple microflora changes throughout the export chain and that apples can become contaminated with foodborne pathogens introduced from various sources. At the starting point in this investigation the source of microflora is the orchard itself, although subsequent sorting, handling, flume water, unclean storage facilities and transport mechanisms can contaminate incoming fruit (Doores, 1983).

Very large standard deviations were recorded throughout the entire export chain, which is due to the complex microbial diversities that are encountered on apple fruit surfaces and the effect of external and internal influences on these populations. Microflora on fruit surfaces can be influenced by geographical area, climatic conditions, pesticide treatments (Vismer *et al.*, 1996), cultivar, physiological status of the individual fruit and different areas on the fruit itself (Doores, 1983). It is advisable that this work should thus be repeated, with more replicates at each point, being a study on its own in order to determine external and internal variable factors.

When unwashed apples were compared to washed apples at the farming level, washed apples yielded smaller total microbial counts and diversities compared to unwashed fruit. Washing of fruit can significantly reduce microbial populations by 1 to 4 log cycles if done properly (Brackett, 1992; Doores, 1983; Spotts and Cervantes, 1993). The average microbial counts for the apples from farm nr.2 were also greater than those of Farm nr.1, possibly because farm nr.2's packinghouse only had one water bath, while farm nr.1's apples went through two washing procedures. Another reason for the decreased counts is

probably because the fruits were kept in a cold room overnight which could result in a decline in total microflora (Doores, 1983; Stekelenburg *et al.*, 2001).

For two of the pallets studied that originated from farm nr.1 (X and Y), an overall increase in microbial counts at the local port (South Africa) was observed. A reason for this increase could be due to temperature increase at the time of loading since the trucks only maintain temperature (personal communication, Colette le Roux, Dole SA) and are unable to cool fruit down. It is known that cold temperatures retard biochemical and physiological processes and inhibit the development of pathogens (Paull, 1999; Stekelenburg *et al.*, 2001). Once these temperatures increase, microbial activity will similarly increase (Doores, 1983). The storage environment (the cold room where the pallets are stored before transport or the trucks themselves) could also have had an effect on the average total counts (Vismer *et al.*, 1996).

After handling of fruit at the local port in South Africa, a general increase in microbial communities was evident for two of the three pallets (X and Z), possibly also due to unsatisfactory storage and transport conditions, as was found in Chapter 3. In this chapter, high microbial counts were found for various surfaces sampled in cold rooms, containers and other areas which export fruit are exposed to. Air-conditioning filters in cold rooms and containers are not often cleaned or replaced so that fungal and bacterial cells can grow in the ductwork and on filters, resulting in increased airborne counts (Stetzenbach and Buttner, 2000) that can settle on fruit (Den Aantrekker *et al.*, 2003). Reused containers should be thoroughly cleaned, kept in good repair, and used only for the transport of uncontaminated food items (Geldreich and Bordner, 1971).

The age of the apples could probably also have had an effect on the fluctuating microbial numbers throughout the export chain. Developing fruit represents a rich environment of nutrients and water, which supports a significant development of microbial activity (Teixidó *et al.*, 1999). Teixidó *et al.* (1999) suggests that as nutrients fluctuate on ripening apple surfaces, there is an ecological succession of bacteria, yeasts and filamentous fungi as seen on leaf surfaces. Additionally, the length of time also

influences microbial populations. Long-term cold storage will further select for psychrotrophic organisms (Brackett, 1992).

Further down the export chain, it was evident that average microbial total counts and diversities followed an interesting unexpected pattern. It was expected that microbial numbers for the fruit would increase from the overseas port to the repacking facilities, followed by an added increase from the repacking facilities to the retail level. These were the anticipated results, since there was an increase in temperature from cold storage to room temperature (APPENDIX A) (Paull, 1999; Stekelenburg *et al.*; 2001) and since there was additional handling of fruit by packing personnel and customers (Ayçiçek *et al.*, 2004; Nel *et al.*; 2004). In contrast, there was no particular trend in microbial dynamics from the European port to the repacking areas, although the counts decreased greatly from the repacking areas to the retail outlets. Further research is needed to clarify this point.

Concerning the total microflora of apples, Teixidó *et al.* (1998) reported that the frequency of bacteria was always less than 5% of the total microbial community, and bacteria were seldom isolated during cold storage. In contrast, yeasts were the predominant component of the total microflora, followed by filamentous fungi (Doores, 1983; Teixidó *et al.*; 1998). This supports part of the findings in our study, but does not explain several cases where bacterial communities dominated the overall microflora.

Postharvest diseases of apples cause major losses, but appeared to be of little concern in this particular study (Table 1 and 2; APPENDIX L). It was surprising that *Penicillium*-like species, particularly *P. expansum*, the major cause of post-harvest apple diseases (Leibinger *et al.*, 1997; Teixidó *et al.*, 1998; Vismer *et al.*, 1996), were never isolated from the apples in our postharvest pathological investigations.

Regarding food safety issues and the presence of *S. aureus* on apples as detected on fruit removed from the chain at different points, it is known that *S.aureus* is toxinogenic and a great cause of food intoxications. It is the first recorded incidence where *Staphylococcus* spp. were isolated from fresh apples, although *S. aureus* and *S. epidermidis* have

previously been isolated from apple juice (Bayindirh *et al.*, 2006; Ghengdesh *et al.*, In Press) and have also been reported in grape, mango, orange and pineapple juice as well as on cabbage, onion, garlic, lettuce, mushrooms, carrots, parsley and radish (Beuchat, 1995; Ghenghesh *et al.*, In Press; Lengeler *et al.*, 1999; Nguyen-the and Carlin, 1994). Human skin and nasal membranes are the main reservoirs of these bacteria, which may contribute to contamination of fruit during distribution and handling if the conditions allow for bacterial growth and toxin production (Johannessen *et al.*, 2002). According to Corbo *et al.* (2004), the presence of *Staphylococcus* spp. from contamination by food handlers in the processing line, would not represent a risk because this microorganism does not compete well with other microorganisms normally present on raw fruit. *Staphylococcus aureus* was mostly present on apples at the South African harbour. Although transport trucks are cool ($\pm 12^{\circ}\text{C}$), they do not reduce the temperature of fruit pallets to the required export temperature of -1.1 to -0.6°C (<http://www.fao.org/DOCREP/005/Y4358E/y4358e04.htm>), but only maintain temperature. In previous studies a significant increase of *S.aureus* was observed when there was a break in the cold chain for more than six hours at 25°C or twelve hours at 15°C (Stekelenburg *et al.*, 2001). *Staphylococcus aureus* does not grow at temperatures below $7-8^{\circ}\text{C}$ (Food Safety and Hygiene, 2000). The specimens of *S. epidermidis* (a pathogen also of human origin) that was found on the fruit at the European port could have been introduced at the port or could have survived the reefer voyage. Last mentioned is highly unlikely, given the fact that the temperature in the reefer was generally below 0°C (APPENDIX M to O).

Quality management consists of a holistic plan that takes all factors responsible for quality losses into account. It then integrates them in such a way that they complement each other. Everyone participating in fruit production, storage and post-harvest handling should be involved since events in the orchard can affect storage life, and incorrect handling during marketing may nullify all previous efforts of quality maintenance (Combrink *et al.*, 1994). A hazard analysis and critical control point program (HACCP) is the best approach to ensure microbial safety in fresh products (Hurst and Schuler, 1992).

Little detailed information exists of the microflora of apples during cold storage. Further research is a prerequisite for understanding and gaining more insight into the effect of the whole export chain on fruit.

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CHAPTER 6

DETERMINING AIR QUALITY IN THE FRUIT CHAIN ENVIRONMENT FROM SOUTH AFRICA TO EUROPE

ABSTRACT

The one potential source of contamination of fresh produce while moving through the export chain is facility air. Contaminated air that settles on fruit can result in quality defects, potential food safety issues and economic loss at the end of the chain if contamination is severe enough. The levels of air microbial contamination for fruit export environments were determined by sampling two farms and a harbour in South Africa and two harbours, two repacking facilities and two retail centres in Europe. Several areas exceeded the maximum acceptable index level of microbial air contamination of 22 cfu/h in food industries. Managing air quality is a critical part of any food safety program and needs to be addressed throughout the entire fruit export environment.

1. INTRODUCTION

Contamination of food products is a primary concern for any processor or manufacturer. Adulteration of fruit can result in loss of quality; affect food safety (Shintani *et al.*, 2004), and ultimately result in economic losses if contamination was severe enough (Den Aantrekker *et al.*, 2003; <http://www.ecolab.com>). Immediately after exposing a product to the environment, it is likely to become contaminated by a wide range of airborne fungi and bacteria that comprise the air spora (Den Aantrekker *et al.*, 2003; Medrela-Kuder, 2003).

The concentration of airborne microorganisms generally depends on factory design, human activity, conditions of general hygiene (Den Aantrekker *et al.*; 2003, Medrela-Kuder, 2003) and prevailing climatic and ecological conditions (Medrela-Kuder, 2003). It has been suggested that airborne bacteria originate from contaminated surfaces or areas

in a processing facility where cleaning and disinfection is minimal (Northcutt *et al.*, 2004). These microorganisms can be transferred through the air while adhering to dust or skin particles and generally settle on surfaces due to gravitation (Den Aantrekker *et al.*, 2003).

Microbial sampling is the most effective approach to determine airborne contaminants that may settle on food or food preparation surfaces (Morris *et al.*, 2000; Pasquarella *et al.*, 2000). Current air sampling relies on two different methods: 1) by active air sampling (with the aid of a mechanical air sampler) which measures air contamination in colony forming units per cubic meter of air (cfu/m³) or 2) passive air sampling using Petri dish settle plates containing solid medium. This method does not permit quantification of particles per unit volume of air but gives an indication of the cfu count per time unit (Morris *et al.*, 2000; Pasquarella *et al.*, 2000; Stetzenbach and Buttner, 2000).

Evaluation of the level of microbial air contamination in places at risk has recently been considered a basic step towards disease prevention (Shintani *et al.*, 2003) and most studies have been carried out in hospitals or healthcare facilities. Current guidelines and standards are focused on combating bio-risks in high-risk environments. The maximum acceptable level or index of microbial air contamination for the food sector is 400 cfu/m³ using the active air sampling method, and 22 cfu/hour (55mm diameter Petri dishes) in the case of the settle plate technique (Pasquarella *et al.*, 2000).

2. MATERIALS AND METHODS

Air samples were taken from the entire apple export chain through which fruit was distributed from two farms in South Africa to retail centres in Europe. Sampling was performed from February till March 2004. The two farms selected were in the same geographical region in the Free State province and were about 20 km apart.

2.1 AIR SAMPLING IN SOUTH AFRICA

In this part of the study, a compact surface air sampler (PBI international, Germany) was used to assess the level of air microbial contamination on two farms and one harbour in

South Africa. Air sampling time was optimised for facility air and cold rooms (unpublished data). The sampler was set at one unit at room temperature and six units in cold storage facilities, thereby absorbing 30 and 180 litres of air per minute at two different units respectively. The air was blown onto 55mm Standard 1 Nutrient Agar (STD1) (Biolab, Johannesburg) and Malt Extract Agar (MEA) (Biolab) contact plates (Bibby Sterilin, UK). Air sampling in the orchard of each farm included two samples per row in five rows. At the packing houses, three samples were taken from the two cold rooms and three samples in the restroom facilities, while six samples were taken in the packing house. At the harbour, three samples were taken in three cold rooms, three samples on three levels in a reefer ship that had just off loaded its fresh fruit cargo, and two samples in each of five containers before and after being washed. Sampling at the harbour was done twice for all the areas, except for the reefer ship. Each sample at every sampling point consisted of two replicates of STD 1 and MEA plates. The contact plates were transported to the lab in a cooler box within 24 hours and prepared for processing.

2.2 AIR SAMPLING IN EUROPE

Air sampling in Europe was done using the passive air sampling method due to breakage of the air sampler while in cargo on route to Europe (poor handling by aircraft staff). Air sampling was performed by exposing 55mm STD1 and MEA contact plates for 30 minutes to the various environments (Pasquarella *et al.*, 2000). These environments included five containers, five trucks and three cold storages on the one harbour; three cold storages and three levels of a ship that had just off loaded its cargo on another harbour; three cold storages and a packing area at two repacking facilities and a holding and display area at two retail centres. In terms of replicates, sampling was performed as described previously.

2.3 SAMPLE PROCESSING

The STD 1 contact plates were incubated for 48h at 25°C and the MEA contact plates for 96h at 25°C. Total counts and specie diversity indexes were recorded and pure cultures were made only from dominant representative isolates (species predominating at each

sampling point throughout the export chain). For the MEA plates, only *Penicillium* spp. were isolated for further identification, because of its association with fruit quality (Agrios, 1997). However, all predominant bacterial species were isolated from STD1 plates, since they could represent potential foodborne pathogens. All pure cultures were preserved by freeze-drying (Dhingra and Sinclair, 1985). The identity of bacterial organisms was based on preliminary assays i.e. colony appearance, cellular morphology, motility, Gram stain, oxidase and catalase reactions as appropriate and the API CH, API Staph, API 20NE (Biomérieux, France) and Biolog GP2 (Biolog, USA) systems. Identification of fungi was done with the aid of R. Jacobs, M. Truter and B. Demoz (Plant Pathology laboratories, Mycological Unit, University of Pretoria).

2.4 ENVIRONMENTAL MONITORING

Temperatures were measured throughout the experiment as described in Chapter 3, Section 2.3.

2.5 STATISTICAL ANALYSIS

As microbial counts are not normally distributed with heterogeneous variances, differences between treatment effects (areas of sampling) were tested using the method known as generalized linear modelling (Dobson, 1983). The gamma distribution best modelled the data obtained in this study (Lefkovitch, 1993). Fishers' protected t-test Least Significant Difference (LSD) was applied to separate means at the 1 % level of significance. In the case of bacteria, numbers greater than 300 and for fungi and yeasts, numbers greater than 200, were pre-processed before analysis as these censored observations were replaced by estimated values, using the method outlined by Taylor (1973). This method estimates the expected value of each censored observation iteratively conditional on the fact that the value must be greater than the fixed bound, and using the relevant information from the other observations in the experiment.

3. RESULTS

Several representative microbial species were identified from the air sampling plates. The area where they were isolated from as well as their known significances are recorded in Table 1.

Microbial counts for both farms were the highest at the packinghouses, which were still within the international norm for food manufacturing facilities. The highest fungal counts for farm nr.1 were recorded in the orchard and packinghouse, although it was not significantly higher than that recorded for cold storage nr.1 (Fig. 1; APPENDIX S). On the second farm, very low microbial counts were found in the orchard but very high fungal counts were recorded in the packinghouse and cold storage nr.2. Bacterial counts were significantly higher in the packinghouse of farm nr.1, while in the orchard of farm nr.2 significantly lower bacterial counts were recorded.

The biggest microbial diversity was recorded in the restroom/toilet facility and the second cold storage room (Fig. 2; APPENDIX S). Bacterial diversities were statistically similar at the various places sampled. Only farm nr.1 will be illustrated, since there were no significant differences for farm nr.2 ($P=0.031$).

Significantly higher bacterial counts were recorded in washed containers (Fig. 3; APPENDIX T) compared to unwashed containers. Fungal counts were lower than the international standard once the containers were washed, although this does not reflect a significant difference with unwashed containers. Population diversities decreased slightly after washing, but were of no significance ($P=0.962$).

Total bacterial densities for the cold storages of farm nr.1 (Fig. 4; APPENDIX U) and farm nr.2 (Fig. 6; APPENDIX U) were significantly higher than for the cold storage facilities at the South African harbour, although it was still well below the international standard for food premises. Fungal and yeast diversities were significantly lower at the South African harbour's cold storages (Fig. 5; APPENDIX U). Microbial diversities for

the cold storages of farm nr.2 compared to that of the harbour did not differ significantly ($P=0.172$).

There were no significant differences between the cold storages of the two European harbours (APPENDIX V) in terms of counts ($P=0.788$) or diversities ($P=0.756$), nor any differences between the two repacking facilities ($P=0.815$ for densities and $P=0.919$ for diversities; APPENDIX W) or the two retail centres ($P=0.627$ for densities and $P=0.808$ for diversities; APPENDIX X).

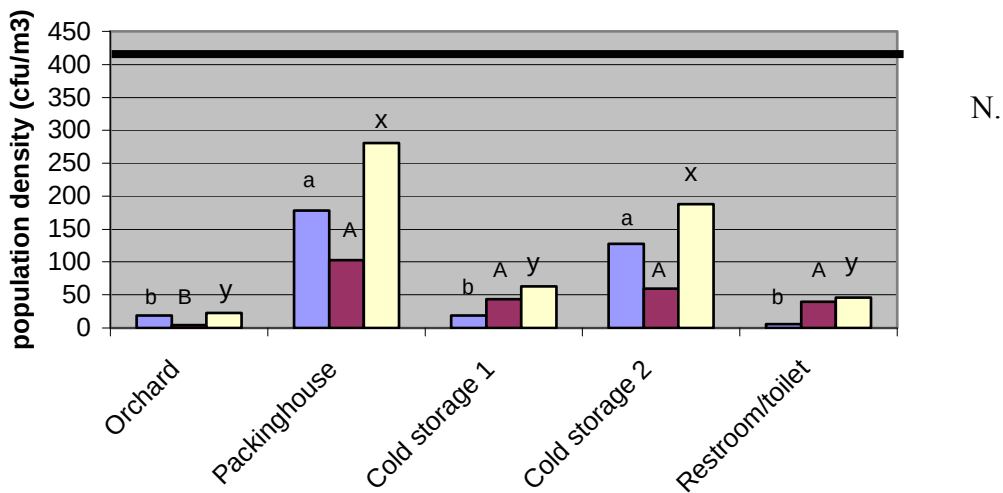
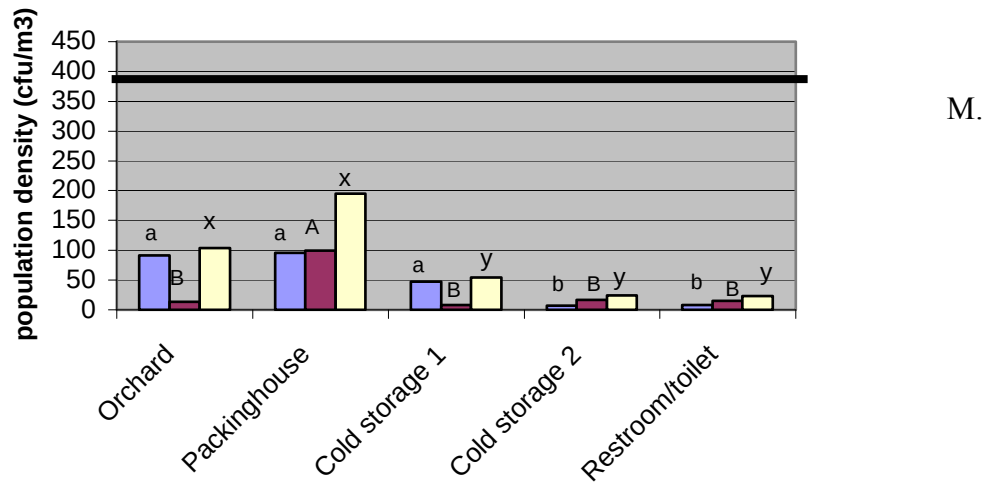


Figure 1 Average microbial total counts from air samples for two farms (M and N). Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level. Population density refers to total number of colonies counted as cfu/m³. The horizontal shaded bar on the y-axis is the standard for food industries (400cfu/m³).
 M= Farm 1
 N= Farm 2

Fungal and yeast counts
 Bacterial counts
 Total microbial count

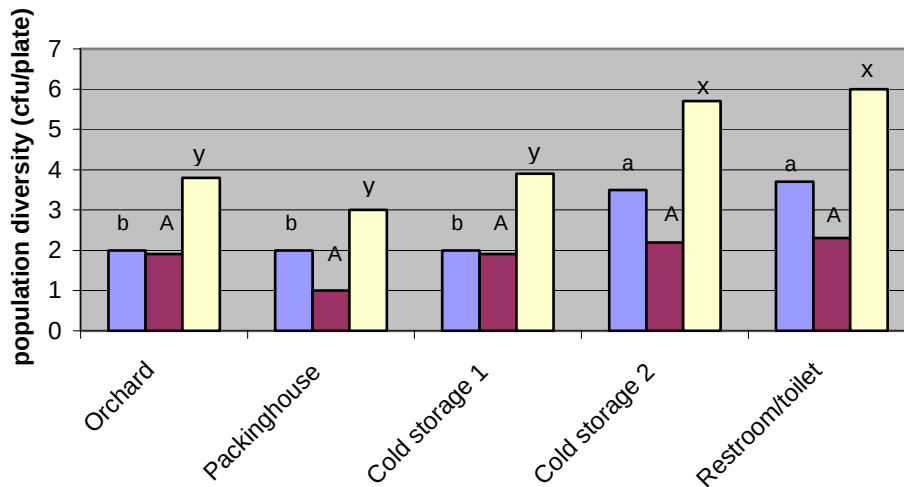


Figure 2 Average microbial diversities of air samples on farm nr.1. Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level.

■ Fungal and yeast diversities
 ■ Bacterial diversities
 ■ Total microbial diversity

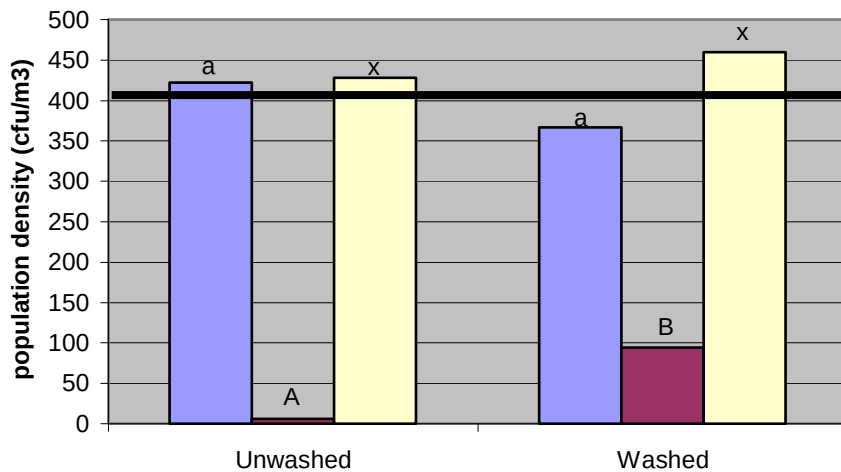


Figure 3 Comparison of average microbial total counts from air samples for unwashed and washed containers on South Africa's harbour. Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level. Population density refers to total number of colonies counted as cfu/m³. The horizontal shaded bar on the y-axis is the standard for food industries (400cfu/m³).

■ Fungal and yeast counts
 ■ Bacterial counts
 ■ Total microbial count

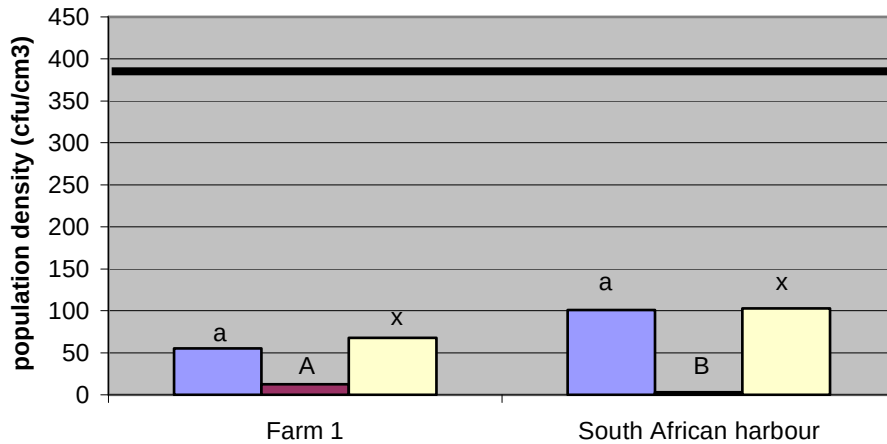


Figure 4 Comparison of average microbial total counts from air samples for the cold storages of the South African harbour and farm nr.1. Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level. Population density refers to total number of colonies counted as cfu/m³. The horizontal shaded bar on the y-axis is the standard for food industries (400cfu/m³).

■ Fungal and yeast counts
 ■ Bacterial counts
 ■ Total microbial count

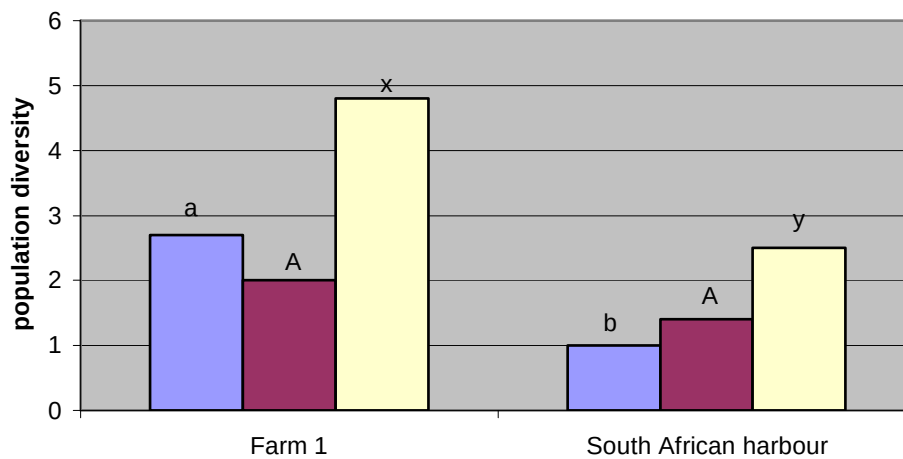


Figure 5 Average microbial diversities of air samples for cold storages of the South African harbour and farm nr.1. Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level.

■ Fungal and yeast diversities
 ■ Bacterial diversities
 ■ Total microbial diversity

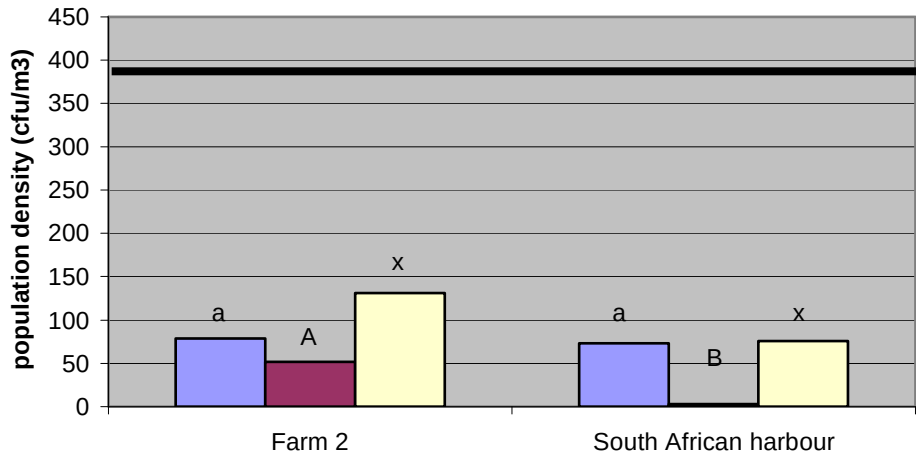


Figure 6 Comparison of average microbial total counts from air samples for the cold storages of the South African harbour and farm nr.2. Bars with the same uppercase letter did not differ significantly at the 1% level. Bars with the same small case letter did not differ significantly at the 1% level. Population density refers to total number of colonies counted as cfu/m³. The horizontal shaded bar on the y-axis is the standard for food industries (400cfu/m³).

Fungal and yeast counts
 Bacterial counts
 Total microbial count

Table 1 Microbial species isolated throughout the ca to Europe

Species isolated	Point of isolation	Known significance	Reference
BACTERIA			
<i>Arthrobacter ilicis</i>	Orchard on farm in South Africa	Usual habitat is soil, described as causing blight on American Holly	Jones and Collins, 1984
<i>Arthrobacter woluwensis</i>	Restroom on farm in South Africa	Usual habitat is soil, not a known pathogen	Jones and Collins, 1984
<i>Bacillus megaterium</i>	Restroom on farm in South Africa	Typical habitat is soil	Sneath, 1984
<i>Brevibacterium casei</i>	Cold room on farm in South Africa	Usual habitat is dairy products, skin, sea fish	Jones and Collins, 1984
<i>Brevibacterium otitidis</i>	Cold room on farm in South Africa	Usual habitat is dairy products, skin, sea fish	Jones and Collins, 1984
<i>Microbacterium maritopicum</i>	Orchard on farm in South Africa	Occurs in dairy products	Jones and Collins, 1984
<i>Micrococcus spp.</i>	Cold room on farm in South Africa	Primary natural habitat is mammalian skin	Schleifer, 1984
<i>Moraxella spp.</i>	Packinghouse on farm in South Africa	Associated with infections (conjunctivitis, abscesses, etc.)	http://freepages.pavilion.net/tetrix/moraxella.html
<i>Pasteurella haemolytica</i>	Cold room on European harbour	Causes pneumonia and “shipping fever” in cattle, sheep and goats	Carter, 1984
<i>Rhodococcus fascians</i>	Cold room on farm in South Africa	Reported as causing leaf gall of many plants and fasciation of sweet peas	Lechevalier, 1984
<i>Sanguibacter keddieii</i>	Truck on South African harbour	Non-pathogenic, terrestrial origin	http://www.phac-aspc-gc.ca/ols-bsl/pathogen/pdf/pathogensfinal-092001.pdf

Table 1 continued



Species isolated	Point of isolation	Known significance	Reference
<i>Staphylococcus capitis</i>	Cold room on farm in South Africa	Habitats include human scalp, forehead, eyebrows, face, neck, ears	Schleifer, 1984
<i>Staphylococcus cohnii cohnii</i>	Restroom on farm in South Africa	Colonizes humans, most strains are multiple antibiotic-resistant	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=12503405&dopt=Abstract
<i>Tsukamurella inchoensis</i>	Packinghouse on farm in South Africa	Has reportedly been isolated from human specimens, associated with clinical disease	http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=11283073
FUNGI			
<i>Penicillium commune</i> Thom	Cold storage on harbour	Of common occurrence in foods and other habitats	Pitt, 1988
<i>Penicillium crustosum</i> Thom	Cold storage at repacking facility	Ubiquitous spoilage fungus; weak pathogen of pomaceous fruits; common biodeteriogen	Pitt, 1988
<i>Penicillium glabrum</i> (Wehmer) Westling	Cold storage on harbour and farm in South Africa	Cosmopolitan; widely distributed in soils; biodeteriorative agent	Pitt, 1988
<i>Penicillium janthinellum</i> Biourge	Cold storage on harbour, cold room at repacking facility	Ubiquitous soil fungus	Pitt, 1988
<i>Penicillium spinulosum</i> Thom	Cold storage on farm in South Africa, truck on harbour	Ubiquitous; commonly occurring in soils, decaying vegetation, foods and textiles; frequently isolated from substrates containing heavy metals, acids and tannins	Pitt, 1988

4. DISCUSSION

In this study, the average microbial total counts for all the areas in Europe proved to be very high (APPENDIX V to X), with the highest counts on average being 829 cfu/30 min, while the maximum acceptable level of index of microbial air contamination in food industries is 22 cfu/h (Pasquarella *et al.*, 2000), using the settle plate technique. Settle plates give a measurement of the airborne microbial populations that settle onto a surface at a given time (Pasquarella *et al.*, 2000). Contaminated air has been shown to influence the quality and shelf life of stored products (Marek *et al.*, 2003) and can be a health hazard for people working in these environments, particularly if they are suffering from allergy problems (Medrela-Kuder, 2003).

All of the total air counts in South Africa were below the active air sampling standard for food industries of 400 cfu/m³ (Pasquarella *et al.*, 2000), except for the containers. Since the air sample data in South Africa was obtained by active air sampling and that of Europe by means of passive air sampling, it would not be scientifically justified to compare these areas. Of note is the fact that most of the samples collected by the first-mentioned procedure are below the food industry standard, but those acquired by passive means were well above the standard (even though the plates were only exposed for half an hour and not an hour as specified by the standard).

On the farms, packing houses had the highest total microbial air counts (Fig.1), while low total counts were recorded for the restrooms. A possible explanation for the overall general lower counts at farm nr.1, compared to that found on farm nr.2 is that farm nr.1's facilities were newly built and therefore had not accumulated microbial loads over time. This is in agreement with findings reported by Hamada and Fujita (2002) who described the fact that fungal contamination of an air-conditioner filter increases as it becomes older.

At the South African harbour, washing of containers did not affect total microbial air loads although bacterial counts increased significantly in the air after the containers were washed. This confirms that bacteria get dislodged from the surface during washing and

can move through spray droplets (Elvers and Lappin-Scott, 2000). High-pressure steam cleaning that reaches temperatures of 70°C is normally used to clean containers. Microorganisms can be transferred to the air by spraying during cleaning, which causes aerosol formation (Den Aantrekker *et al.*, 2003). After washing, the containers had lower fungal total counts, although this was not significant. Fungal growth was visible on silicon fasteners in containers, even when the fasteners were replaced shortly before the sampling of unwashed and washed containers was done. Air-conditioning filters are also not often cleaned or replaced so that fungal and bacterial cells can grow in the ductwork and on filters, resulting in increased airborne counts (Stetzenbach and Buttner, 2000). Containers are reusable and should therefore be thoroughly cleaned, kept in good repair, and used only for the transport of uncontaminated food items (Geldreich and Bordner, 1970). Currently there is no traceability system in place to determine what kind of products are transported in the unit.

Packing and display areas at the repacking facilities and retail centres (Appendices W and X respectively) yielded high microbial air counts. Human activity results in increased numbers of airborne bacteria, and coughing for example, is a major means of dispersal and transport of upper respiratory tract flora into the air (Stetzenbach and Buttner, 2000). Brackett (1992) found that handling at the retail level is maybe one of the weakest links in the distribution system, because many retailers are well known for abstaining from proper refrigeration temperatures and good sanitation practices (Brackett, 1992; Paull, 1999).

In general, microbial counts were lower in cold storage facilities (APPENDIX A), probably owing to the fact that cold temperatures retard biochemical and physiological processes, inhibiting the development of pathogens (Paull, 1999; Stekelenburg *et al.*, 2002) and have less turbulence in the air. Relative humidity levels below 70% can also prevent microbial growth (http://www.pwngsc.gc.ca/rps/aes/content/iaq_pub_fungal-e.html).

In most cases, fungal and yeast counts were recorded in higher numbers, compared to bacteria. Fungi are mostly disseminated by air currents that carry them as inert particles

over various distances (Agrios, 1997). They release reproductive spores ranging in size from 2 to 200 μm into the environment (Stetzenbach and Buttner, 2000) and depending on the air turbulence and velocity, the spores may be carried upward or horizontally (Agrios, 1997), enabling them to be dispersed through air currents. Similar to other studies, *Cladosporium* spp. were commonly cited (Stetzenbach and Buttner, 2000) while the spores of numerous other allergenic fungi, belonging to the *Penicillium*-group were also often found. *Penicillium* species are known for causing severe postharvest diseases on citrus and apple fruits and are often associated with decaying plant material (Pitt, 1988). Concerning the bacteria that were picked up throughout the export chain, many of them belong to the Gram-positive cocci (like *Micrococcus* and *Staphylococcus* sp.). According to Stetzenbach and Buttner (2000), these genera comprise approximately 85-90% of culturable microorganisms in air samples. Gram-positive cells are frequently isolated because they have thick cell walls and many contain carotenoid pigments that increase their survival in the air. Several of them, however, are normal flora of the skin and mucous membranes so they are present in occupied indoor environments without associative disease (Stetzenbach and Buttner, 2000).

Under conditions of stress or disturbance, microbial diversity is markedly low, while stable conditions are normally associated with higher species diversity (Atlas and Bartha, 1998). This was evident in the case where farm nr.1's cold storages yielded significantly higher microbial diversities than for the harbour. The farm is a more stable environment where only one commodity is handled, while cold storages at the harbour tend to contain different produce at the same time. Species diversity tends to be low in physically controlled ecosystems and higher in biologically accommodated ecosystems (Atlas and Bartha, 1998).

Facility air is a critical component of any quality or food safety program and controlling contamination by airborne microorganisms is therefore of utmost importance (<http://www.ecolab.com>). This can be achieved by an effective cleaning program (Hamada and Fujita, 2002).

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CHAPTER 7

DETERMINING TOTAL MICROBIAL LOADS THROUGHOUT THE APPLE EXPORT CHAIN. A STATISTICAL ANALYSIS.

ABSTRACT

One of the factors that determine fruit quality is its microbial flora. Fruit carry a natural non-pathogenic epiphytic microflora but can become contaminated with pathogenic microorganisms during export, causing either postharvest decay or possibly resulting in a phytosanitary risk. In order to study microbial dynamics on fruit surfaces and the environment fruit moves through in the export chain, fruit washings were made, surfaces were sampled and total populations determined per cm². Due to biological variation an alternative approach to analyse the data was taken using individual samples in order to better understand microbial behaviour. Comparing this method with standard approaches of data analysis, different results could be obtained, thereby more effectively indicating critical control points throughout the export chain.

1. INTRODUCTION

The ultimate objective of the production, handling and distribution of fresh fruit is to satisfy consumers, and it is generally agreed that consumer satisfaction is related to product quality (Harker *et al.*, 2003, Shewfelt, 1999). Quality in itself can be viewed as the absence of defects, a degree of excellence (Shewfelt, 1999) and the level of product acceptance by consumers (Sloof *et al.*, 1996). Quality and safety of fresh produce depends, amongst other factors, on the composition of microbial flora (European Commission, 2002). For minimally processed fresh fruits and vegetables, mesophilic bacterial total counts on plate count agar have been highly variable and range from 10³ to 10⁶ cfu/g. Product quality is often still acceptable, despite such high counts (Nguyen-the and Carlin, 1994). Although no standard has been set for fresh fruit and the environments

it is exposed to, international standards specify that microbial counts on food-processing equipment should be $<5 \text{ cfu/cm}^2$ in evaluating cleaning efficacy (Dancer, 2004).

When analysing biological data, standard statistical methods allow one to obtain an estimate (mean/average) of some characteristic of a large population from a sample. For the whole population it is likely to follow a normal distribution, if it were plotted. The spread of the curve depends on the variability in the population, being greater if the variability is large (an estimate of variability of the values from one individual to another is known as the standard deviation). This is a common shape of distribution for measurements like length and weight. However, not all measurements/populations follow the normal distribution (Watt, 1994), and such data can be transformed to follow a normal distribution with stabilized variances (log-transformation is an example of this) (Krzanowski, 1998). Differences between areas of sampling are also sometimes tested using the method known as generalized linear modelling (Dobson, 1983; Krzanowski, 1998), as is the case for microbial counts (Watt, 1994). Microbial counts are not normally distributed and have heterogeneous variances (Dobson, 1983).

One method of decreasing variability in an experiment is by following the standard advice of statisticians, who prefer to increase the number of replicates to reduce standard deviation (Watt, 1994). According to Tijskens *et al.* (2003) reproducible results are indeed obtained when this line of thinking is pursued, but the discrepancy between observed and true values was still in the order of magnitude of 100% when colour development of tomatoes was analysed. The research conducted by Tijskens *et al.* (2003) is based on an alternative approach to deal with the variation in properties and quality attributes in all products and batches. The important concept behind biological variance is that each individual (each apple, each sample) has its own level of properties. "...in research as well as in daily practice, we have to avoid working with mean behaviour altogether to advance knowledge and understanding of product behaviour and quality optimisation. Although based entirely on the properties of individuals, biological variance is a property of a population. Attention should therefore be devoted to the behaviour of individuals rather than to the entire population as a whole..." (Tijskens *et al.*, 2003). The individual is thus the important factor, not the mean of the individuals.

Also, by calculating the log values from data, a lot of information on the distribution and biological variance of the data is lost. If one considers that the total colony forming units (cfu) are actually measured, including some measuring error which strongly depends on the dilution factor, by taking the logarithm of cfu (for purposes of ease of working and understanding during transformation), the actual information on the variation and the error is mutilated and lost completely as is indicated in the following equation.

$$\log(cfu + \varepsilon_{actual}) \neq \log cfu + \log(\varepsilon_{imaginary})$$

The aim of this investigation was to analyse previously obtained data of total microbial loads on apples and the environments apples moved through in the export chain from South Africa to Europe (Chapters 3 and 5). This study was therefore conducted with the purpose to evaluate a different statistical approach in order to identify areas of contamination, or the environment and areas that could impact on fruit quality.

2. MATERIALS AND METHODS

2.1 COLLECTION OF DATA

2.1.1 Fruit

Data collection was done as described in Chapter 5 at various points from the farm in South Africa to the supermarket in Europe. Data processing was further described and individual values from each data set used in this study.

2.1.2 Fruit environment

Data collection was done as described in Chapter 3, where several surface samples of the export environment from the farm to the supermarket was obtained. Data processing was described and individual values from each data set used in this study.

2.2 AN ALTERNATIVE APPROACH TO STATISTICAL ANALYSIS

An alternative approach to statistically analyse the data was based on work done by Dr Tijskens at the University of Wageningen. Microbial counts were placed in a limited number (mostly 7) of equidistant separate bins (using a histogram analysis toolpack of Microsoft Excel), each bin having its own numerical boundary. If a microbial count lies within a bin's specific boundary, that count 'falls into' that bin. For example, when 500000-1000000 is a bin, a count like 700000 falls into that bin. When five different counts fall between 500000-1000000, there is a frequency of five. Percentages of each frequency, relative to the total number of samples used, were calculated to enable comparisons between locations. To eventually plot a graph, fungal/yeast counts were combined with bacterial counts (to increase the number of samples in a graph) and this total microbial count was plotted against percentages of frequencies. This was done to see whether a similar growth trend (curve shape) could be observed for different environments throughout the export chain.

3. RESULTS

For the environmental data collected throughout the export chain, a consistent curve shape could be observed for the environments for all three traced pallets. This shape could be ascribed to the fact that most of the total counts lay in the 0-3000 000 range, while the minority of the counts was between 3000 000-9500 000 (Fig. 1 to 3). The information on the environment from South Africa's harbour on to the retail centres in Europe is the same for pallet 1 of farm nr.1 and for the pallet of farm nr.2. For pallet 2 of farm nr.1, the environment was the same up to the European port, but differed from the repack facility to the retail centre in Europe.

Concerning the actual fruit data, a graph could not be compiled for all separate samples because of an insufficient amount of useful data points. In contrast with the environmental data, the apple washings were the only source of information and data could not be pooled as with the different environmental surfaces (like brushers, rollers, etc) that together comprised one location (the packinghouse). The values for the

microbial loads on all available apple samples were however, orders of magnitude smaller than for the surroundings (about 0-10000 cfu/cm²) (Fig. 5).

In figure 4, total microbial counts for unwashed versus washed containers on South Africa's harbour were compared. The microbial loads for both were almost alike, and a similar curve shape as for figures 1 to 3 was obtained.

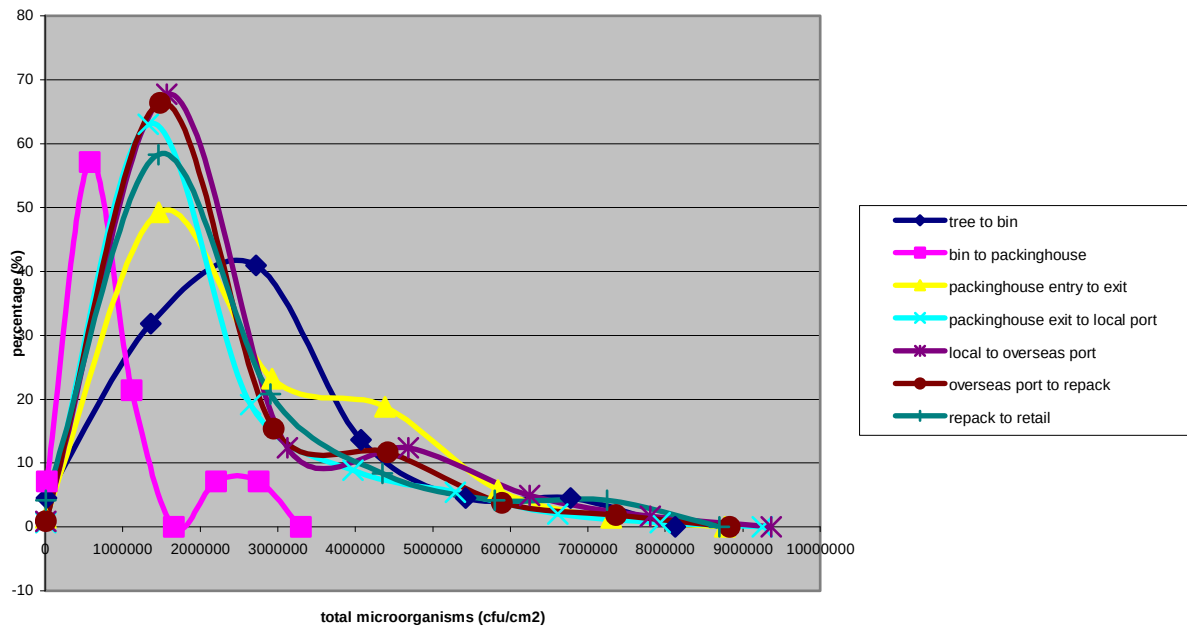


Figure 1 Total microbial counts for the export environment of the first pallet from farm 1.

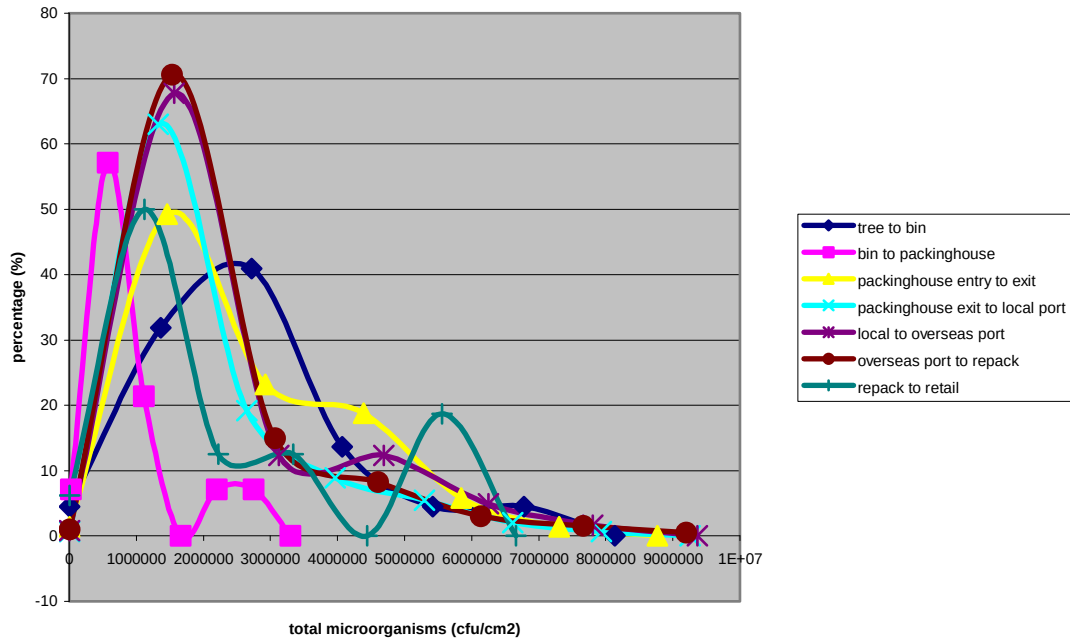


Figure 2 Total microbial counts for the export environment of the second pallet from farm 1.

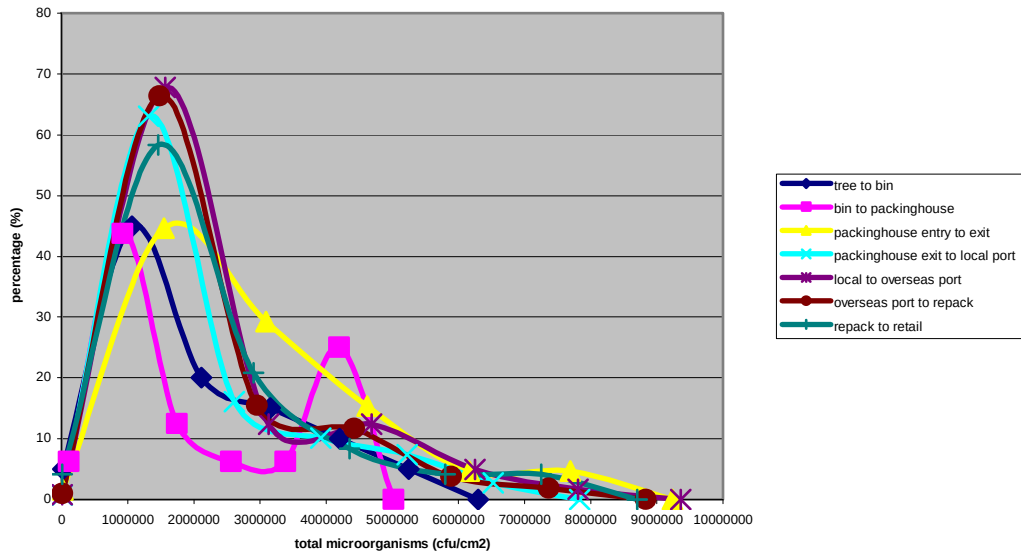


Figure 3 Total microbial counts for the export environment of the pallet from farm 2.

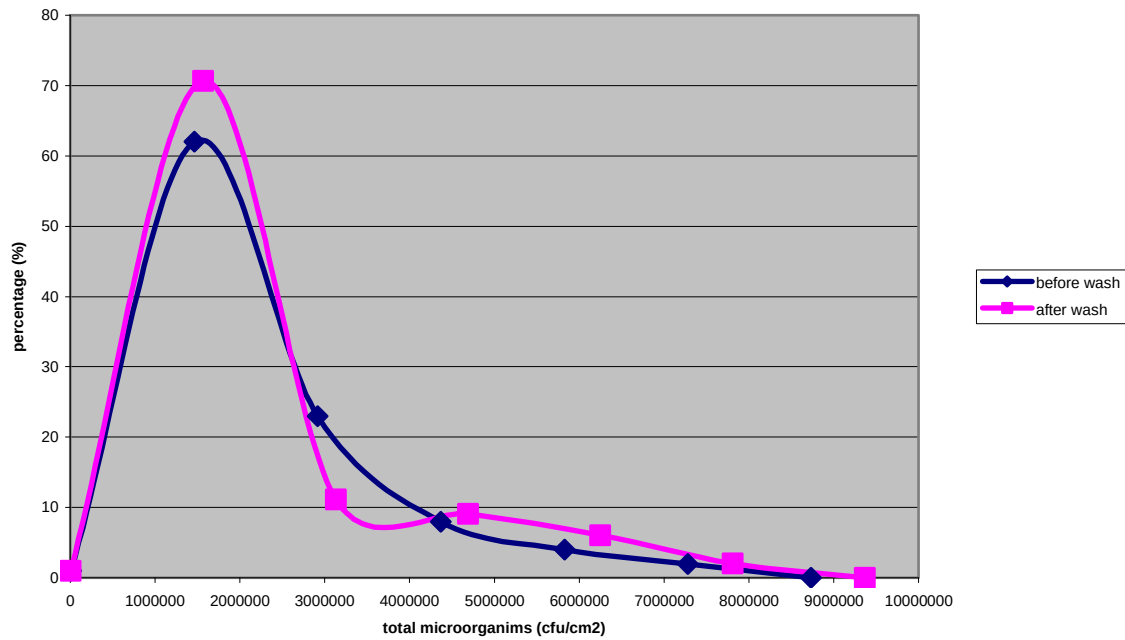


Figure 4 Total microbial counts for washed and unwashed containers on South Africa's harbour.

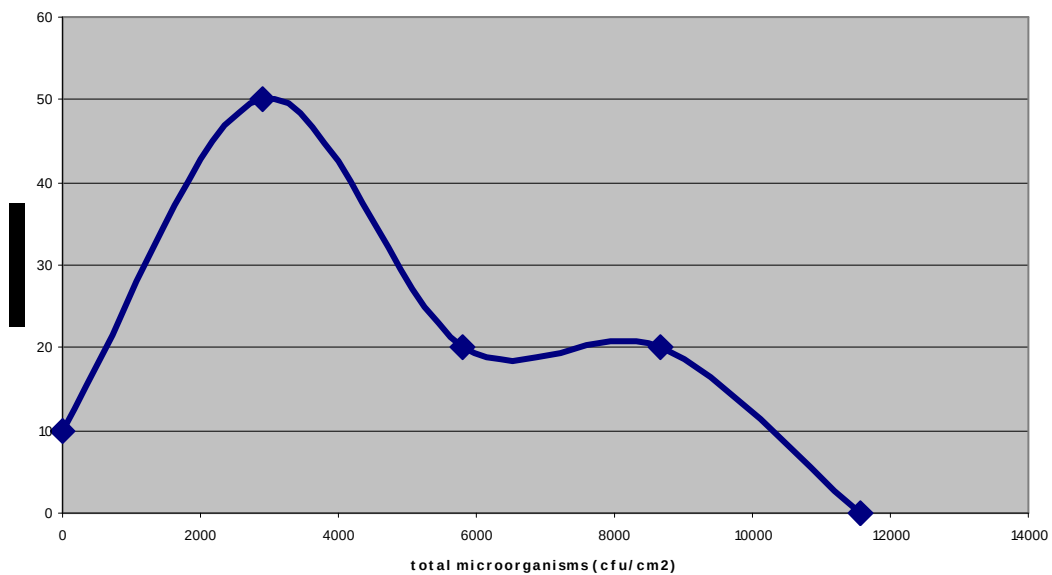


Figure 5 Total microbial counts on apples on the tree in the orchard of farm 2.

4. DISCUSSION

In this investigation it was found that all environments that export fruit moved through, exceeded the international hygiene standard for cleaning efficacy of food-processing equipment which is $<5 \text{ cfu/cm}^2$ (Dancer, 2003). This is mainly because total microbial counts were generally in the 0-3000 000 cfu/cm^2 range. Absence of high percentage of postharvest apple losses (Chapter 5) could prove that the environment (with the majority total counts in the $0-10^6$ range) did not have a negative impact on the microbial fruit quality in this investigation. These limited values are supported by Vismer *et al.* (1996). We therefore believe that a standard of $<5 \text{ cfu/cm}^2$ as is used in the food-processing industry is far too strict for environments through which fresh fruit moves and that a more realistic standard should be proposed.

From the data analysed with the approach of Dr Tijskens, it was evident that most of the microbial counts in all environments evaluated, lay between 0 and 3000 000, indicating that the environments were reasonably clean. Few samples had higher counts than 3000 000 cfu/cm^2 (between 3000 000 and 9500 000) and these included areas like brushers and rollers in the packinghouses, air conditioning systems in the cold rooms, etc. These areas could represent true critical control points where hygiene standards exceed acceptable norms and should therefore require more frequent cleaning.

Completely different conclusions could be drawn from the alternative approach as opposed to the standard method of analysis. The distribution of the curves can be compared to the shape of the distribution of colour difference in apples (APPENDIX Y), based on a similar logistic function as for the growth of microorganisms (Tijskens *et al.*, 2003). When apples are immature, the majority of the fruit fell within the 20% total colour range. In time (during storage), the distribution becomes less skewed, flatter and wider and at the end of the storage period, the distribution becomes skewed again, but this time to the right (tailing to the left), when all the apples are yellow. Similarly, in the case of microbial loads, when the microbial populations are “young” (recently established) (most left curves of Fig. 1 to 4), the majority of the populations fall within the 40-70% range. If the curve would shift completely to the right, the microbial populations would have reached their maximum microbial loads per surface area. A

microbial community would, however, never reach the maximum stage, since microbial populations within the community interact/compete with each other (Atlas and Bartha, 1998). The shifting of the curves from left to right as time passes is analogous to a “time-bomb” situation: the most left curve has less potential to “explode” than the centre curves. Hereby, it is implied that in time, as the total microbial populations increase, they can reach their ultimate peak of population density per surface area that could represent a potential hazard. In this investigation it was observed that the curves for total microbial loads were all skewed to the left and never reached the right side of the graph. Apart from interactions within a microbial community, a reason for this could be the hygiene practices used throughout the export chain that prevent the microbial populations from reaching their maximum density and therefore becoming skewed to the right. In figures 1 to 3, the curves for the packinghouses (packinghouse entry to exit) were the furthest from the left (the packinghouses had the highest frequency of counts in the $>3000\ 000\ \text{cfu/cm}^2$ region).

It is proposed that in future a variety of a single type location analyses in the export chain be studied (e.g. packinghouses). By obtaining samples from these locations at different seasons for a longer investigation period, while observing initial and final microbial loads and environmental parameters in time, a better understanding of the overall microbial dynamics would follow.

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CHAPTER 8

GENERAL DISCUSSION

Apples are amongst the most important deciduous tree fruits (Jackson, 2003) and have remained a major export commodity (Groenewald, 2000). In this study, the individual and combined effects of the production, handling and distribution of apples on the subsequent microbial contamination level during the entire export chain were analysed.

Of the six well-known foodborne pathogens i.e. *Shigella sonnei*, *Salmonella muenchen*, *Listeria monocytogenes*, *Staphylococcus epidermidis*, *Escherichia coli* and *Staphylococcus aureus* that were investigated for their occurrence in the apple export chain environment, only the latter two were isolated. Pathogenic *S. aureus* is one of the most common causing agents of food intoxication (Frazier and Westhoff, 1988) and was obtained from containers on a port in Europe and in the display area of a supermarket. Together with *S. epidermidis*, it was also isolated from apple surfaces as reported for the first time. *Escherichia coli*, a potentially highly resistant pathogen commonly occurring in faeces, was present in large numbers in fruit washing water on a farm in South Africa. Although pathogens like *S. sonnei*, *S. muenchen* and *L. monocytogenes* have been frequently isolated from fresh produce (Beuchat, 2002; Brackett, 1992; Untermann, 1998) and surfaces like floor drains, walls, (Brackett, 1992), hands (Kusumaningrum *et al.*, 2002) and water (De Roever, 1999), they were not detected in this study.

In this investigation it was found that the total microflora on apple fruit surfaces fluctuate during the export process. It was also found that common postharvest fungal pathogens (Leibinger *et al.*, 1997; Teixidó *et al.*, 1998; Vismer *et al.*, 1996) did not cause major losses. All the surfaces that exported apples were exposed to, exceeded the internationally recognized standard of cleaning efficacy (Dancer, 2004). In this study it was found that washing of containers did not have a significant impact on reducing microbial load. Further, this study indicated that the standard of cleaning efficacy is too strict for fresh fruit handling systems, since the current hygiene standard is only applicable in food-processing environments and it is therefore advisable that a more realistic guideline be

developed. A thorough literature search for total microbial load standards for fruit chain environments yielded no results. The only useful information obtained, was that of Vismer *et al.* (1996), who stated that fungal loads of larger than 10^5 indicate infected or damaged apples, thereby implying that an environment with fungal counts in this range, could possibly lead to infected fruit if these microorganisms were to be transferred. Also, at Agrotechnology and Food Innovations (Wageningen), an informal standard of 10^5 - 10^7 microorganisms exists for cut fruit and vegetable environments (personal communication, W. de Heij, A&F). The environment, with the majority total counts in the 0 - 10^6 range, did not have a negative impact on the microbial fruit quality in this investigation. Therefore a standard of 10^5 total microorganisms for apple export chain environments can be proposed.

Contamination of air has been shown to influence the shelf life of stored products (Marek *et al.*, 2003). In this study, two types of air sampling methods have been used to determine the air quality throughout the export chain. Average total air microbial counts in Europe proved to be well above the maximum acceptable level of 22 cfu/h (Pasquarella *et al.*, 2000) using the settle plate technique, while most of the areas sampled in South Africa were below the standard recommended when using the active air sampling method.

Statistical analysis is an important tool to interpret biological data. When an alternative statistical analysis method was applied in this study to compare data sets, completely different conclusions could be drawn from this approach as opposed to the standard method of analysing. With the standard method, average means are used to describe microbial behaviour. By handling the data as individual samples, a different picture emerged. Microbial counts in all environments lay between 0 and 3000 000 while few samples had higher counts than 3000 000 cfu/cm². These mainly included areas like brushers and rollers in the packinghouses and air conditioning systems in the cold rooms. These areas could represent true control points in a HACCP program where hygiene standards exceed international norms.

The insufficient data obtained with the apple microflora studies raised the question to whether the fruit investigation was approached from the right angle. Generally the counts were low (0-10000 cfu/cm²), even when the apples passed through environments that had higher microbial loads. Possible explanations could be the probability that apples have active anti-microbial properties, or that the microorganisms could not attach successfully due to the waxy surface of apples, or even that the microorganisms were mainly in a spore-form and were subsequently not picked up. This brings us to the point to whether microbial load indeed affects fruit quality? According to Nguyen-the and Carlin (1994), total bacterial counts of minimally processed fruits and vegetables at the end of storage are unrelated to sample quality. In total, few fruit had defects (the highest value at one stage was 8%) and these were caused by endophytes. In future studies, it could be considered to pass apples through environments with high microbial loads and observe what the effect is on microbial growth and apple quality. Hereby, the dynamic resistance of the apples to highly contaminated environments can be examined, enabling us to predict how a particular apple cultivar will react to different export situations. Emphasis therefore changes from the environment to the product and how to produce fruit with a stable climax community that can resist changes in export environments.

Regarding species diversity, it was found to be lower on physically disturbed surfaces and areas containing less fresh commodities as opposed to facilities where different commodities are regularly handled. Stable conditions are normally associated with higher species diversity (Atlas and Bartha, 1998).

The survival of foodborne and postharvest plant pathogens on fresh produce varies according to the microbial population involved and the conditions under which the produce is handled and stored during production and shipment purchase (Madden, 1992). Control of microbiological diseases is an integrated, holistic process that starts in the orchard and is concluded in the packinghouse and storage facilities. All factors affecting apple fruit quality should be taken into account in order to develop an integrated system of postharvest quality management (Combrink *et al.*, 1994).

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SUMMARY

FOOD SAFETY AND QUALITY THROUGHOUT THE APPLE EXPORT CHAIN

by

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One of the factors that maintains fruit quality is its microbial flora. Fruit holds a natural non-pathogenic epiphytic microflora but can become contaminated with pathogenic microorganisms during export, causing either postharvest decay or possibly resulting in a food safety risk. In order to study microbial dynamics on fruit surfaces and the environment fruit moves through in the export chain, fruit washings were made, surfaces were sampled and total populations and diversities determined per cm². Hygiene and safety levels for fruit export environments were hereby determined by sampling various points along the apple export chain, which included two farms and a harbour in South Africa and two harbours, two repacking facilities and two retail centres in Europe.

In this first study of its kind, all the surfaces that were sampled exceeded the international standard for cleaning efficacy of food-processing equipment that is <5 cfu/cm², while several areas exceeded the maximum acceptable index level of microbial air contamination of 22 cfu/h in food industries. Washing of containers on a harbour in South Africa did not have a significant impact on microbial populations. Regarding fruit quality, it was determined that apple microflora fluctuate throughout the export process and that postharvest pathogens that are known to cause great economic losses in the apple industry, proved to be of little significance in this investigation.

The presence of six foodborne pathogens i.e. *Shigella sonnei*, *Salmonella muenchen*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* was monitored throughout the chain. Of these, only *S. aureus* and *E. coli* were recorded, although pathogenicity was not confirmed for the latter. *Staphylococcus*

aureus was found in containers and at a retail centre in Europe, and *S. aureus* and *S. epidermidis* were recorded on apple surfaces for the first time. *Escherichia coli* was present in great numbers in fruit washing water on a farm in South Africa.

Since the standard for food premises is very stringent and perhaps inapplicable for fresh fruit handling and holding facilities, future research should include development of a more realistic hygiene standard for fresh fruit environments.

APPENDIX A Average temperatures observed throughout the apple export chain

Location		Average temperature (°C)
FARM 1	Orchard	27.8
	Packinghouse	20.8
	Cold room 1	9.4
	Cold room 2	11.9
	Restroom	18.0
FARM 2	Orchard	25.2
	Packinghouse	24
	Cold room 1	13.2
	Cold room 2	8.0
	Restroom	26.2
SOUTH AFRICAN HARBOUR	Cold room 1	-0.1
	Cold room 2	0.2
	Cold room 3	0.0
	Outside	24.3
	Containers (unwashed)	21.8
	Containers (washed)	20.1
	Trucks	19.6
	Ship	6.4
EUROPEAN HARBOUR 1	Cold room 1	8.1
	Cold room 2	9.7
	Cold room 3	1.3
	Outside	13.4
	Ship	13.7
EUROPEAN HARBOUR 2	Cold room 1	1.1
	Cold room 2	0.0
	Cold room 3	4.6
	Containers	9.9
	Trucks	11.7
REPACKING FACILITY 1	Outside	10.5
	Cold room 1	7.3
	Cold room 2	0.3
	Cold room 3	3.3
	Packing area	9
REPACKING FACILITY 2	Outside	14.4
	Cold room 1	9.2
	Cold room 2	2.1
	Cold room 3	1.7
	Packing area	8.2
RETAIL CENTRE 1	Outside	7.8
	Storage room	8.0
RETAIL CENTRE 2	Display area	9.5
	Storage room	5.0
	Display area	8.7

APPENDIX B Average microbial counts and diversities for farms (followed by the standard deviation) (NA=not applicable)

FUNGI AND YEASTS					
Sampling area	n	Farm 1		Farm 2	
		Population density	Population diversity	Population density	Population diversity
Air-con	8	753125 ± 891471	0.75 ± 0.36	4525250 ± 5656236	0.80 ± 0.27
Fruit bins	5	3440000 ± 2850088	0.53 ± 0.29	8140000 ± 4461278	0.29 ± 0.15
Bin covers	5	NA	NA	12536711 ± 21389483	0.54 ± 0.43
Brushers	4	37626241 ± 39058930	0.28 ± 0.09	41510150 ± 18206867	0.29 ± 0.12
Unused boxes	5	17990274 ± 30191224	0.38 ± 0.35	2740400 ± 2614441	0.58 ± 0.39
Flaps	4	2375000 ± 1403282	0.48 ± 0.35	3785500 ± 5050842	0.65 ± 0.42
Pallet bases	5	19008968 ± 29399320	0.38 ± 0.36	22749357 ± 25942244	0.21 ± 0.06
Walls	12	4675583 ± 4160409	0.45 ± 0.35	3550000 ± 4615842	0.39 ± 0.38
Packers' hands	5	16491331 ± 30790421	0.70 ± 0.41	1300000 ± 2241651	0.84 ± 0.37
Pickers' hands	5	34053872 ± 34137955	0.50 ± 0.45	5540000 ± 5207975	1.12 ± 1.65
Unused pulp trays	5	388000 ± 514315	0.67 ± 0.47	720000 ± 1285302	0.44 ± 0.52
Rolers	4	20413511 ± 34092521	0.69 ± 0.37	31211896 ± 22727516	0.57 ± 0.50
Picking bags	5	45624983 ± 35365237	0.35 ± 0.19	1220000 ± 1080278	0.41 ± 0.34
Table surfaces	4	7525000 ± 6908618	0.64 ± 0.41	27634732 ± 26718346	0.36 ± 0.43
Toilet taps	4	19165234 ± 34866890	0.23 ± 0.16	6300600 ± 3726392	0.40 ± 0.34
Floors	12	14441898 ± 18318024	0.37 ± 0.23	3658333 ± 1722818	0.25 ± 0.15
Conveyer belts	4	20024031 ± 34386044	0.79 ± 0.43	NA	NA
Sizers	4	5125000 ± 3790646	0.15 ± 0.01	28006724 ± 26232041	0.38 ± 0.16
<i>F probability</i>		0.006	0.089	0.021	0.281

APPENDIX B continue

BACTERIA					
		Farm 1		Farm 2	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Air-con	8	28636380 ± 51057810	0.47 ± 0.44	24553454 ± 44081742	0.42 ± 0.42
Fruit bins	5	8820000 ± 6394294	0.41 ± 0.11	13620000 ± 4024550	0.40 ± 0.34
Bin covers	5	NA	NA	23358143 ± 37712067	0.34 ± 0.06
Brushers	4	104613625 ± 97181788	0.35 ± 0.10	90476412 ± 0.01	0.16 ± 0.06
Unused boxes	5	78796546 ± 100000000	0.22 ± 0.13	12020000 ± 6832789	0.26 ± 0.10
Flaps	4	145585504 ± 86190487	0.25 ± 0.02	26873898 ± 42452326	0.18 ± 0.08
Pallet bases	5	151635055 ± 82866157	0.32 ± 0.19	5900000 ± 1630951	0.49 ± 0.33
Walls	12	29925247 ± 57613367	0.37 ± 0.30	11160833 ± 8045558	0.34 ± 0.24
Packers' hands	5	83473298 ± 96095786	0.48 ± 0.32	26794020 ± 36001389	0.28 ± 0.14
Pickers' hands	5	83013584 ± 96745026	0.30 ± 0.13	73105973 ± 38911438	0.26 ± 0.09
Unused pulp trays	5	40858665 ± 82735724	0.43 ± 0.32	5720000 ± 4788208	0.44 ± 0.33
Rolers	4	57245788 ± 87633898	0.27 ± 0.12	70947797 ± 39032038	0.34 ± 0.44
Picking bags	5	79715845 ± 99514321	0.24 ± 0.10	5620000 ± 3208894	0.27 ± 0.19
Table surfaces	4	144662968 ± 88042035	0.19 ± 0.06	73720526 ± 33409579	0.29 ± 0.06
Toilet taps	4	51222880 ± 91677151	0.32 ± 0.08	10380000 ± 10385567	0.57 ± 0.40
Floors	12	34215462 ± 44144082	0.33 ± 0.16	22754642 ± 21019515	0.25 ± 0.08
Conveyer belts	4	52447412 ± 90992857	0.15 ± 0.02	NA	NA
Sizers	4	188687927 ± 0.01	0.30 ± 0.07	48047980 ± 49134509	0.67 ± 0.39
<i>F probability</i>		0.477	0.550	0.021	0.162

APPENDIX B continue

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
		Farm 1		Farm 2	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Air-con	8	29389506 ± 51292300	1.22 ± 0.71	29078705 ± 49645745	1.22 ± 0.56
Fruit bins	5	12260001 ± 8865833	0.94 ± 0.24	21760001 ± 4660129	0.69 ± 0.28
Bin covers	5	NA	NA	35894856 ± 39647194	0.88 ± 0.46
Brushers	4	142239867 ± 1.1 x 10 ⁸	0.63 ± 0.09	131986564 ± 18206867	0.45 ± 0.07
Unused boxes	5	96786821 ± 1.2 x 10 ⁸	0.61 ± 0.34	14760401 ± 8407913	0.85 ± 0.42
Flaps	4	147960505 ± 85448815	0.73 ± 0.34	30659400 ± 47058474	0.83 ± 0.36
Pallet bases	5	170644025 ± 94923127	0.69 ± 0.32	28649358 ± 25495686	0.70 ± 0.35
Walls	12	34600831 ± 59970826	0.81 ± 0.46	14710834 ± 11753297	0.74 ± 0.52
Packers' hands	5	99964629 ± 1.2 x 10 ⁸	1.18 ± 0.66	28094021 ± 35375133	1.11 ± 0.31
Pickers' hands	5	117067457 ± 78908808	0.80 ± 0.37	78645974 ± 41330376	1.38 ± 1.65
Unused pulp trays	5	41246666 ± 83042760	1.1 ± 0.32	6440001 ± 5649602	0.87 ± 0.54
Rolers	4	77659300 ± 1.2 x 10 ⁸	0.96 ± 0.35	102159694 ± 53017921	0.92 ± 0.43
Picking bags	5	125340829 ± 1.3 x 10 ⁸	0.59 ± 0.19	6840001 ± 3256993	0.67 ± 0.35
Table surfaces	4	152187970 ± 81269305	0.83 ± 0.41	101355260 ± 54723852	0.64 ± 0.41
Toilet taps	4	70388115 ± 87130362	0.55 ± 0.13	16680601 ± 13469595	0.98 ± 0.30
Floors	12	48657361 ± 55600360	0.70 ± 0.37	26412977 ± 21425452	0.51 ± 0.16
Conveyer belts	4	72471444 ± 87391075	0.93 ± 0.43	NA	NA
Sizers	4	193812928 ± 3790646	0.45 ± 0.07	76054705 ± 75191755	1.05 ± 0.28
<i>F probability</i>		0.364	0.096	0.009	0.330

APPENDIX C Average microbial counts and diversities for unwashed and washed containers on the South African harbour, followed by the standard deviation

FUNGI AND YEASTS						
Sampling area	Before wash			After wash		
	n	Population density	Population diversity	n	Population density	Population diversity
Walls	30	4977433 ± 6585439	0.53 ± 0.38	27	3703704 ± 6284903	0.58 ± 0.42
Silicon fasteners	30	10408729 ± 8924293	0.52 ± 0.33	27	9532670 ± 8905785	0.53 ± 0.37
Floors	30	11201489 ± 9317027	0.47 ± 0.32	27	9076674 ± 9033161	0.35 ± 0.33
<i>F probability</i>		0.938	0.399			
BACTERIA						
Sampling area	Before wash			After wash		
	n	Population density	Population diversity	n	Population density	Population diversity
Walls	30	15861922 ± 14126571	0.43 ± 0.34	27	15685587 ± 15041277	0.39 ± 0.35
Silicon fasteners	30	23511752 ± 11422347	0.40 ± 0.30	27	18338361 ± 14224275	0.39 ± 0.32
Floors	30	19612425 ± 12128067	0.40 ± 0.30	27	19097519 ± 11510430	0.28 ± 0.18
<i>F probability</i>		0.894	0.611			

APPENDIX C continue

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)						
Sampling area	Before wash			After wash		
	n	Population density	Population diversity	n	Population density	Population diversity
Walls	30	20839357 ± 17946030	0.96 ± 0.48	27	19389291 ± 17867568	0.98 ± 0.62
Silicon fasteners	30	33920482 ± 17443050	0.92 ± 0.42	27	27871032 ± 21647633	0.92 ± 0.46
Floors	30	30813915 ± 15200987	0.87 ± 0.44	27	28174194 ± 17013818	0.63 ± 0.38
<i>F probability</i>		0.954	0.275			

APPENDIX D Average microbial counts and diversities for the cold storage facilities of South Africa's harbour, followed by the standard deviation (±SD)

Sampling area	n	FUNGI AND YEASTS		BACTERIA	
		Population density	Population diversity	Population density	Population diversity
Air-con	18	6195624 ± 9152158	0.82 ± 0.35	10727360 ± 14525839	0.17 ± 0.47
Flaps	18	7549988 ± 9745204	0.44 ± 0.42	12616554 ± 12348684	0.17 ± 0.18
Walls	18	8952584 ± 9892876	0.58 ± 0.40	12002797 ± 14146731	0.13 ± 0.18
Plastic sheets	18	11076081 ± 10599528	0.77 ± 0.38	13913945 ± 14409372	0.15 ± 0.13
Floors	18	12573412 ± 8866792	0.65 ± 0.41	21239220 ± 12310158	0.37 ± 0.66
<i>F probability</i>		0.867	0.035	0.857	0.367

APPENDIX D continue

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)			
Sampling area	n	Population density	Population diversity
Air-con	18	16922985 ± 18548585	0.99 ± 0.63
Flaps	18	20166543 ± 17348199	0.62 ± 0.50
Walls	18	20955382 ± 18511348	0.71 ± 0.41
Plastic sheets	18	24990027 ± 17808425	0.92 ± 0.37
Floors	18	33812633 ± 19594897	1.02 ± 0.67
<i>F probability</i>		0.713	0.098

APPENDIX E Average microbial counts and diversities for the cold storage facilities of European harbour 1, followed by the standard deviation (±SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Walls	9	4206950 ± 7130428	0.63 ± 0.37	14684981 ± 16656230	0.42 ± 0.35
Floors	9	5340871 ± 9213577	0.78 ± 0.33	15995361 ± 15709233	0.28 ± 0.16
<i>F probability</i>		0.795	0.375	0.916	0.297

APPENDIX E continue

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)			
Sampling area	n	Population density	Population diversity
Walls	9	18891932 ± 20737647	1.04 ± 0.27
Floors	9	21336233 ± 20664946	1.06 ± 0.39
<i>F probability</i>		0.853	0.938

APPENDIX F Average microbial counts and diversities for the cold storage facilities of European harbour 2, followed by the standard deviation (±SD)

FUNGI AND YEASTS				BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Air-con	9	10725076 ± 12001250	0.49 ± 0.39	15892755 ± 24518564	0.41 ± 0.34
Used boxes	5	1063000 ± 1294257	0.53 ± 0.43	89226971 ± 45239363	0.40 ± 0.34
Walls	9	3734493 ± 7141498	0.81 ± 0.37	16303539 ± 29217289	0.75 ± 0.40
Used pulp trays	5	3066000 ± 3634144	0.36 ± 0.37	23836695 ± 47901983	0.51 ± 0.46
Floors	9	4236367 ± 7275644	0.57 ± 0.41	21934424 ± 22381466	0.41 ± 0.24
<i>F probability</i>		0.295	0.290	0.457	0.225

APPENDIX F continue

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)			
Sampling area	n	Population density	Population diversity
Air-con	9	26617832 ± 28108184	0.90 ± 0.44
Used boxes	5	90289972 ± 45845392	0.93 ± 0.66
Walls	9	20038033 ± 33985291	1.56 ± 0.43
Used pulp trays	5	26902696 ± 51396498	0.87 ± 0.73
Floors	9	26170792 ± 27321237	0.98 ± 0.41
<i>F probability</i>		0.308	0.050

APPENDIX G Average microbial counts and diversities for the ships on South Africa and Europe's harbour, followed by the standard deviation

FUNGI AND YEASTS						
South African harbour				European harbour		
Sampling area	n	Population density	Population diversity	n	Population density	Population diversity
Walls	9	4454634 ± 7384037	0.53 ± 0.45	9	1333889 ± 1254392	0.61 ± 0.40
Floors	9	12753281 ± 9913123	0.53 ± 0.24	9	9419870 ± 9740483	0.54 ± 0.45
<i>F probability</i>		0.338	0.786			

APPENDIX G continue

BACTERIA						
Sampling area	South African harbour			European harbour		
	n	Population density	Population diversity	n	Population density	Population diversity
Walls	9	17069273 ± 13775340	0.35 ± 0.14	9	18853587 ± 16377423	0.37 ± 0.38
Floors	9	15560502 ± 12073525	0.36 ± 0.28	9	27132335 ± 12392740	0.26 ± 0.14
<i>F probability</i>		0.493	0.448			
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)						
Sampling area	South African harbour			European harbour		
	n	Population density	Population diversity	n	Population density	Population diversity
Walls	9	21523908 ± 16726028	0.88 ± 0.45	9	20187477 ± 16986760	0.98 ± 0.32
Floors	9	28313784 ± 19329770	0.89 ± 0.28	9	36552206 ± 20000250	0.80 ± 0.55
<i>F probability</i>		0.629	0.471			

APPENDIX H Average microbial counts and diversities for repacking facility 1 in Europe, followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Packers' hands	5	4500000 $\pm$ 2290197	0.41 $\pm$ 0.35	50054696 $\pm$ 91409518	0.52 $\pm$ 0.45
Walls	13	5696900 $\pm$ 8315648	0.50 $\pm$ 0.37	30311331 $\pm$ 63352979	0.42 $\pm$ 0.42
Floors	13	10673099 $\pm$ 11992498	0.37 $\pm$ 0.30	131022737 $\pm$ 86076710	0.41 $\pm$ 0.35
<i>F probability</i>		0.326	0.611	0.280	0.869
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Packers' hands	5	54554697 $\pm$ 91865663	0.93 $\pm$ 0.47		
Walls	13	36008232 $\pm$ 66015150	0.92 $\pm$ 0.49		
Floors	13	141695837 $\pm$ 91589847	0.78 $\pm$ 0.43		
<i>F probability</i>		0.079	0.706		

APPENDIX I Average microbial counts and diversities for repacking facility 2 in Europe, followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Air-con		12722293 $\pm$ 17386777	0.72 $\pm$ 1.37	29196604 $\pm$ 25554452	0.35 $\pm$ 0.23
Unused boxes		30769851 $\pm$ 38530507	0.51 $\pm$ 0.45	25447362 $\pm$ 40716090	0.44 $\pm$ 0.33
Packers' hands		35619464 $\pm$ 34457220	0.39 $\pm$ 0.36	45869011 $\pm$ 48176758	0.19 $\pm$ 0.12
Walls		4606674 $\pm$ 10318915	0.44 $\pm$ 0.40	16682332 $\pm$ 28475604	0.48 $\pm$ 0.37
Floors		20808107 $\pm$ 21110424	0.41 $\pm$ 0.35	36238071 $\pm$ 26111300	0.22 $\pm$ 0.07
<i>F probability</i>		0.220	0.853	0.685	0.069
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Air-con		41918898 $\pm$ 29514911	1.07 $\pm$ 1.35		
Unused boxes		56217214 $\pm$ 72219111	0.95 $\pm$ 0.39		
Packers' hands		81488476 $\pm$ 82633528	0.57 $\pm$ 0.28		
Walls		21289006 $\pm$ 30589377	0.92 $\pm$ 0.53		
Floors		57046179 $\pm$ 39668627	0.63 $\pm$ 0.33		
<i>F probability</i>		0.210	0.596		

APPENDIX J Average microbial counts and diversities for retail centre 1 in Europe, followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Customers' hands	5	48480406 $\pm$ 42848571	0.48 $\pm$ 0.48	127610445 $\pm$ 1.1 x 10 ⁸	0.48 $\pm$ 0.47
Walls	7	1751429 $\pm$ 3168912	0.61 $\pm$ 0.37	23868440 $\pm$ 44899889	0.55 $\pm$ 0.34
Floors	7	17948784 $\pm$ 20020989	0.29 $\pm$ 0.32	139975886 $\pm$ 90211973	0.30 $\pm$ 0.32
<i>F probability</i>		0.023	0.308	0.156	0.458
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Customers' hands	5	176090852 $\pm$ 1.5 x 10 ⁸	0.96 $\pm$ 0.40		
Walls	7	25619869 $\pm$ 45833394	1.16 $\pm$ 0.60		
Floors	7	157924671 $\pm$ 94645655	0.59 $\pm$ 0.64		
<i>F probability</i>		0.110	0.200		

APPENDIX K Average microbial counts and diversities for retail centre 2 in Europe, followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Customers' hands	5	74936928 $\pm$ 62249498	0.68 $\pm$ 0.44	259718367 $\pm$ 0.01	0.54 $\pm$ 0.43
Walls	7	11577269 $\pm$ 26459970	0.60 $\pm$ 0.39	65280832 $\pm$ 1.0 $\times 10^8$	0.52 $\pm$ 0.36
Floors	7	72940562 $\pm$ 52546170	0.30 $\pm$ 0.32	140941608 $\pm$ 1.2 $\times 10^8$	0.38 $\pm$ 0.43
<i>F probability</i>		0.179	0.198	0.406	0.731
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Customers' hands	5	334655296 $\pm$ 62249498	1.22 $\pm$ 0.53		
Walls	7	76858102 $\pm$ 1.2 $\times 10^8$	1.11 $\pm$ 0.63		
Floors	7	213882171 $\pm$ 1.4 $\times 10^8$	0.68 $\pm$ 0.47		
<i>F probability</i>		0.259	0.201		

APPENDIX L Quality survey of South African apples performed at the European harbour (courtesy of Maria del Carmen de Unio).

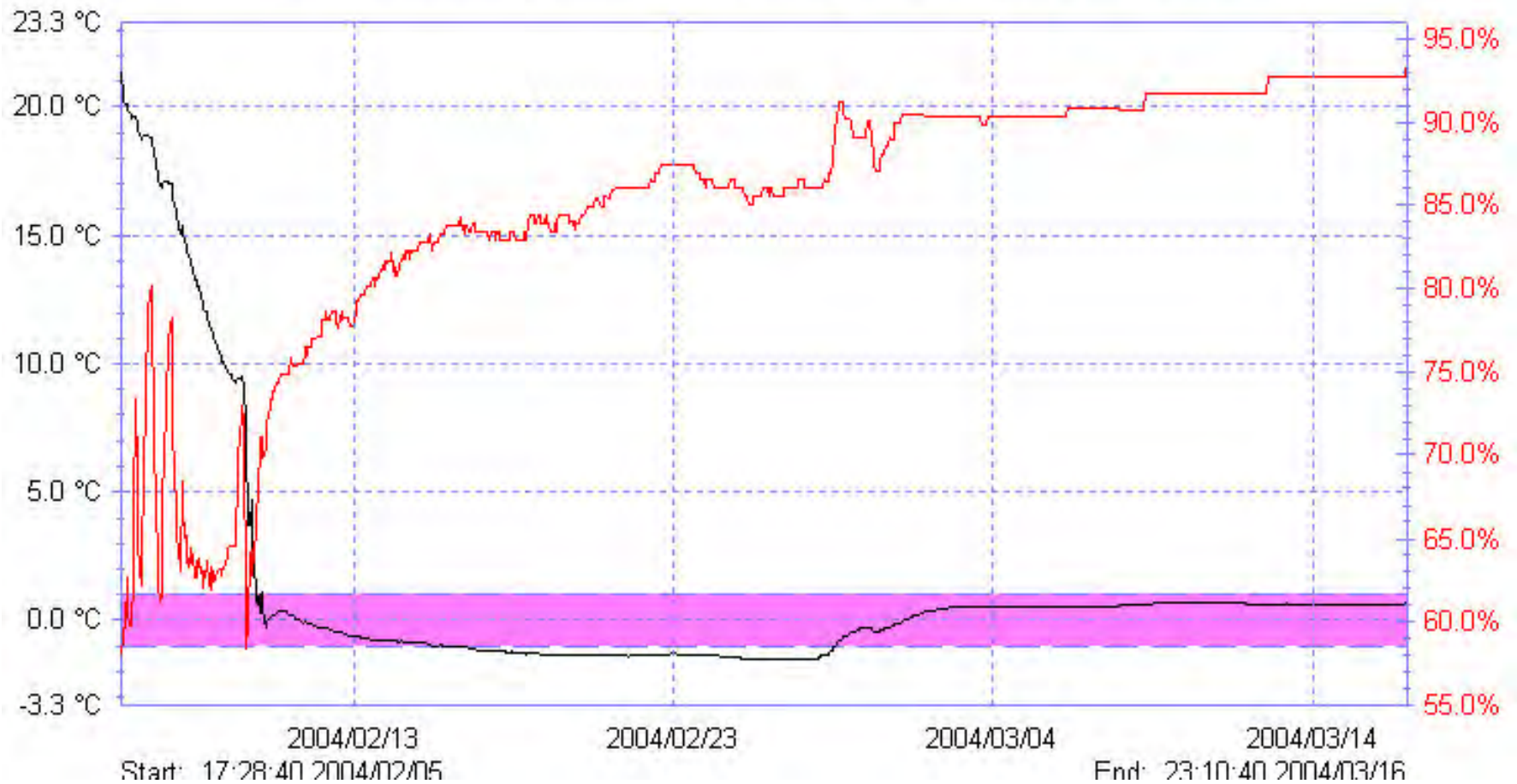
CODE*	mechanical damage	serious shriveling	open injury	bruises	decay	Pressure (kg/cm²)	Brix sugar content
Farm (1) 1.1	2	29	-	-	-	8.5	15
Farm (1) 1.2	1	17	-	1	1	7.5	14
Farm (1) 1.3	2	9	-	-	-	8	12
Farm (1) 1.4	3	23	-	-	-	7.5	13
Farm (1) 1.5	2	15	-	-	1	8.5	13
Farm (1) 1.6	2	10	-	-	-	13	14
Farm (1) 1.7	-	11	-	1	1	11	14
Farm (1) 1.8	2	5	-	-	-	12	12
Farm (1) 1.9	3	13	-	1	-	11	14
Farm (1) 1.10	2	18	-	-	1	13	15
Farm (1) 1.11	2	-	-	-	-	13.5	11.5
Farm (1) 1.12	-	-	-	2	-	13	12
Farm (1) 1.13	2	-	-	1	-	11	12
Farm (1) 1.14	3	-	-	1	-	12.5	11.5
Farm (1) 1.15	1	-	-	2	1	14.5	12
Farm (1) 2.1	2	12	-	-	-	7.5	13
Farm (1) 2.2	1	28	-	-	-	8.5	13
Farm (1) 2.3	2	9	-	-	1	6.5	13
Farm (1) 2.4	3	23	-	-	-	7	14
Farm (1) 2.5	-	32	-	-	-	9.5	14
Farm (1) 2.6	1	9	-	-	1	9	12
Farm (1) 2.7	1	9	-	-	-	10	13
Farm (1) 2.8	1	5	-	-	1	10.5	12
Farm (1) 2.9	1	11	-	-	-	9	13
Farm (1) 2.10	2	12	-	-	-	11.5	14
Farm (1) 2.11	2	12	-	-	-	7.5	13
Farm (1) 2.12	1	28	-	-	-	8.5	13
Farm (1) 2.13	2	9	-	-	1	6.5	13
Farm (1) 2.14	3	23	-	-	-	7	14
Farm (1) 2.15	-	37	-	-	-	9.5	14
Farm (2) 1.1	2	22	2	-	-	7.5	13
Farm (2) 1.2	1	9	-	-	1	8	14
Farm (2) 1.3	2	16	-	-	-	7	14
Farm (2) 1.4	1	7	-	-	-	8.5	14
Farm (2) 1.5	1	19	2	-	-	7	14
Farm (2) 1.6	2	15	2	-	-	9	14
Farm (2) 1.7	1	2	-	-	1	12.5	14
Farm (2) 1.8	-	9	-	-	-	9.5	14
Farm (2) 1.9	1	2	-	-	-	11	14
Farm (2) 1.10	1	3	2	-	-	11	13
Farm (2) 1.11	-	-	2	1	-	11.5	12.5
Farm (2) 1.12	-	-	1	1	-	14	13
Farm (2) 1.13	1	-	-	-	-	13	14
Farm (2) 1.14	1	-	-	-	-	13	13
Farm (2) 1.15	2	-	1	-	-	13	12.5

*The first number in the code represents the pallet number, while the second number indicates a box in the pallet. Fifteen boxes per pallet were analysed. The value of each measurement is out of 85 apples.

TempTaleH: Ambient + Humidity

Label S/N: 127258

Monitor S/N: 161930417



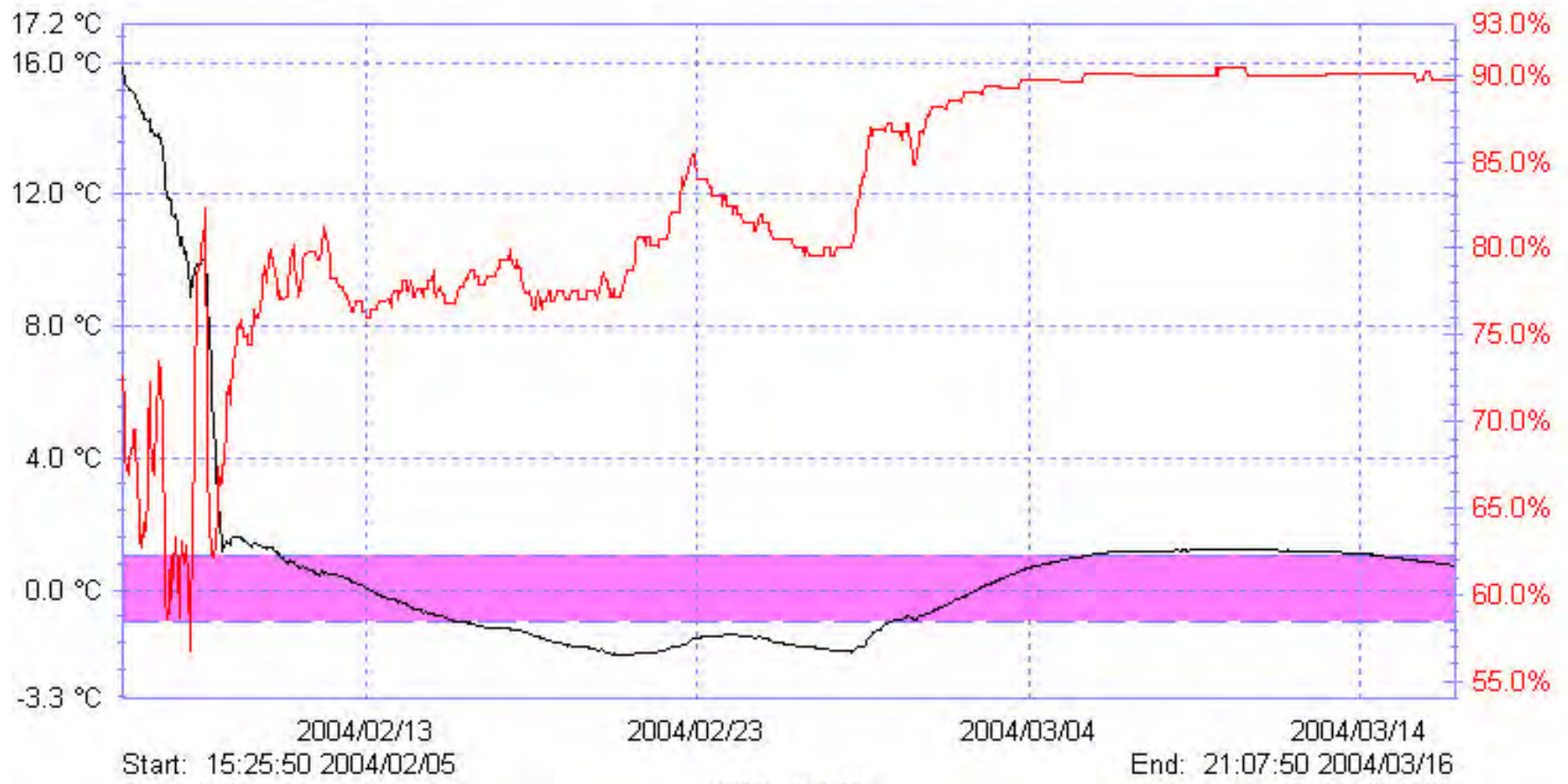
APPENDIX M

Temperature and relative humidity readings from the temperature logger for the fruit pallet from farm 2 in South Africa to the European port. Measurements were taken from 5/2/2004 to 16/4/2004.

TempTaleH: Ambient + Humidity

Label S/N: 127257

Monitor S/N: 161631836



APPENDIX N

Temperature and relative humidity readings from the temperature logger for the first fruit pallet from farm 1 in South Africa to the European port. Measurements were taken from 5/2/2004 to 16/4/2004.

TempTaleH: Ambient + Humidity

Label S/N: 127259

Monitor S/N: 161930406



APPENDIX O

Temperature and relative humidity readings from the temperature logger for the second fruit pallet from farm 1 in South Africa to the European port. Measurements were taken from 5/2/2004 to 16/4/2004.

APPENDIX P Average microbial counts and diversities for fruit collected from the first pallet of farm 1 followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Tree	5	382600 $\pm$ 684725	0.62 $\pm$ 0.38	54200 $\pm$ 52471	0.73 $\pm$ 0.38
Bin	5	42400 $\pm$ 52600	0.62 $\pm$ 0.36	34000 $\pm$ 24083	0.69 $\pm$ 0.43
Packinghouse entry	5	54000 $\pm$ 45056	1.00 $\pm$ 0.01	13000 $\pm$ 9566	0.60 $\pm$ 0.29
Packinghouse exit	5	6200 $\pm$ 7981	0.72 $\pm$ 0.32	32600 $\pm$ 39036	0.70 $\pm$ 0.41
Local port	5	353400 $\pm$ 342108	0.73 $\pm$ 0.37	138000 $\pm$ 93915	0.84 $\pm$ 0.36
Overseas port	5	452400 $\pm$ 812212	0.52 $\pm$ 0.44	1820000 $\pm$ 3846037	0.83 $\pm$ 0.38
Repacking facility	5	369000 $\pm$ 692806	0.68 $\pm$ 0.44	866000 $\pm$ 1241088	0.66 $\pm$ 0.46
Retail centre	3	15333 $\pm$ 13317	0.99 $\pm$ 0.01	40000 $\pm$ 51962	1.00 $\pm$ 0.01
<i>F probability</i>		0.011	0.397	<0.001	0.863
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area		Population density		Population diversity	
Tree		436800 $\pm$ 712046		1.35 $\pm$ 0.59	
Bin		76400 $\pm$ 58333		1.31 $\pm$ 0.75	
Packinghouse entry		67000 $\pm$ 40143		1.60 $\pm$ 0.29	
Packinghouse exit		38800 $\pm$ 37533		1.42 $\pm$ 0.48	
Local port		491400 $\pm$ 419333		1.57 $\pm$ 0.40	
Overseas port		2272400 $\pm$ 4655749		1.35 $\pm$ 0.70	
Repacking facility		1235000 $\pm$ 1155162		1.35 $\pm$ 0.68	
Retail centre		55333 $\pm$ 43096		1.99 $\pm$ 0.01	
<i>F probability</i>		<0.001		0.749	

APPENDIX Q Average microbial counts and diversities for fruit collected from the second pallet of farm 1 followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	N	Population density	Population diversity	Population density	Population diversity
Tree	5	382600 $\pm$ 684725	0.62 $\pm$ 0.38	54200 $\pm$ 52471	0.73 $\pm$ 0.38
Bin	5	42400 $\pm$ 52600	0.62 $\pm$ 0.36	34000 $\pm$ 24083	0.69 $\pm$ 0.43
Packinghouse entry	5	54000 $\pm$ 45056	1.00 $\pm$ 0.01	13000 $\pm$ 9566	0.60 $\pm$ 0.29
Packinghouse exit	5	6200 $\pm$ 7981	0.72 $\pm$ 0.32	32600 $\pm$ 39036	0.70 $\pm$ 0.41
Local port	5	202400 $\pm$ 186598	0.86 $\pm$ 0.31	830000 $\pm$ 1605304	0.83 $\pm$ 0.38
Overseas port	5	94400 $\pm$ 67207	0.62 $\pm$ 0.36	90000 $\pm$ 72111	1.00 $\pm$ 0.01
Repacking facility	5	404000 $\pm$ 520653	1.00 $\pm$ 0.01	786000 $\pm$ 1414355	1.00 $\pm$ 0.01
Retail centre	3	45667 $\pm$ 14887	0.73 $\pm$ 0.43	9000 $\pm$ 8544	0.67 $\pm$ 0.58
<i>F probability</i>		0.007	0.239	<0.001	0.480
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area		Population density		Population diversity	
Tree		436800 $\pm$ 712046		1.35 $\pm$ 0.59	
Bin		76400 $\pm$ 58334		1.31 $\pm$ 0.75	
Packinghouse entry		67000 $\pm$ 40143		1.60 $\pm$ 0.29	
Packinghouse exit		38800 $\pm$ 37533		1.42 $\pm$ 0.48	
Local port		1032400 $\pm$ 1612452		1.69 $\pm$ 0.43	
Overseas port		184400 $\pm$ 109439		1.62 $\pm$ 0.36	
Repacking facility		1190000 $\pm$ 1381883		2.00 $\pm$ 0.01	
Retail centre		54667 $\pm$ 39310		1.39 $\pm$ 0.49	
<i>F probability</i>		<0.001		0.359	

APPENDIX R Average microbial counts and diversities for fruit collected from the pallet of farm 2 followed by the standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	N	Population density	Population diversity	Population density	Population diversity
Tree	5	96000 $\pm$ 71274	0.56 $\pm$ 0.46	102800 $\pm$ 65873	0.78 $\pm$ 0.31
Bin	5	526800 $\pm$ 709387	0.38 $\pm$ 0.21	1106000 $\pm$ 1629018	0.44 $\pm$ 0.44
Packinghouse entry	5	1720800 $\pm$ 2565853	0.64 $\pm$ 0.35	2002000 $\pm$ 2207940	0.52 $\pm$ 0.44
Packinghouse exit	5	270000 $\pm$ 426204	0.55 $\pm$ 0.42	562000 $\pm$ 815598	0.41 $\pm$ 0.34
Local port	5	180000 $\pm$ 83666	1.00 $\pm$ 0.01	172000 $\pm$ 137550	0.88 $\pm$ 0.26
Overseas port	5	566000 $\pm$ 927351	0.52 $\pm$ 0.44	638000 $\pm$ 1320871	0.88 $\pm$ 0.26
Repacking facility	5	230800 $\pm$ 285242	0.90 $\pm$ 0.23	1094400 $\pm$ 2184903	0.74 $\pm$ 0.37
Retail centre	3	10000 $\pm$ 2000	0.75 $\pm$ 0.24	26333 $\pm$ 29160	0.84 $\pm$ 0.29
<i>F probability</i>		0.079	0.101	0.228	0.174
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area		Population density		Population diversity	
Tree		198800 $\pm$ 64399		1.34 $\pm$ 0.52	
Bin		1632800 $\pm$ 2293818		0.82 $\pm$ 0.61	
Packinghouse entry		3722800 $\pm$ 4494107		1.16 $\pm$ 0.64	
Packinghouse exit		832000 $\pm$ 1231909		0.95 $\pm$ 0.49	
Local port		352000 $\pm$ 210998		1.88 $\pm$ 0.26	
Overseas port		1204000 $\pm$ 1554992		1.40 $\pm$ 0.61	
Repacking facility		1325200 $\pm$ 2450898		1.64 $\pm$ 0.35	
Retail centre		36333 $\pm$ 29263		1.59 $\pm$ 0.51	
<i>F probability</i>		0.064		0.047	

APPENDIX S Average microbial air counts and diversities for farms (followed by standard deviation)

FUNGI AND YEASTS					BACTERIA				
Sampling area	n	Farm 1 Population density ¹	Population diversity ³	Farm 2 Population density ¹	Population diversity ³	Farm 1 Population density ²	Population diversity ⁴	Farm 2 Population density ²	Population diversity ⁴
Orchard	20	91 ± 107 a	2.0 ± 0.5 b	19 ± 50 b	1.7 ± 1.2 a	13 ± 8 b	1.9 ± 0.9 a	4 ± 7 b	1.3 ± 0.7 a
Packinghouse	12	96 ± 114 a	2.0 ± 0.8 b	178 ± 120 a	1.6 ± 1.1 a	99 ± 99 a	1.0 ± 0.5 a	103 ± 122 a	0.7 ± 0.7 a
Cold storage 1	6	47 ± 95 a	2.0 ± 1.1 b	19 ± 6 b	1.7 ± 0.9 a	8 ± 3 b	1.9 ± 0.5 a	44 ± 21 a	1.6 ± 0.4 a
Cold storage 2	6	7 ± 2 b	3.5 ± 0.7 a	128 ± 130 a	1.8 ± 1.1 a	17 ± 7 b	2.2 ± 1.2 a	60 ± 17 a	1.1 ± 0.3 a
Restroom/toilet	6	8 ± 4 b	3.7 ± 1.2 a	6 ± 3 b	2.9 ± 1.3 a	15 ± 7 b	2.3 ± 0.8 a	40 ± 29 a	1.6 ± 1.0 a
<i>F probability</i>		<0.001	<0.001	<0.001	0.259	<0.001	0.012	<0.001	0.073

TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Farm 1 Population density	Population diversity	Farm 2 Population density	Population diversity
Orchard	20	104 ± 107 a	3.8 ± 1.2 b	23 ± 53 b	3.0 ± 1.1 a
Packinghouse	12	195 ± 114 a	3.0 ± 0.6 b	281 ± 191 a	2.3 ± 1.4 a
Cold storage 1	6	55 ± 93 b	3.9 ± 1.1 b	64 ± 20 b	3.3 ± 0.8 a
Cold storage 2	6	24 ± 8 b	5.7 ± 1.4 a	188 ± 146 a	2.9 ± 1.3 a
Restroom/toilet	6	23 ± 10 b	6.0 ± 0.9 a	46 ± 30 b	4.4 ± 1.5 a
<i>F probability</i>		<0.001	<0.001	<0.001	0.031

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

¹ A combined analysis of the two farms indicated no significant differences (P=0.171)

² A combined analysis of the two farms indicated no significant differences (P=0.717)

³ A combined analysis of the two farms indicated no significant differences (P=0.018)

⁴ A combined analysis of the two farms indicated significant differences (P<0.001)

APPENDIX T Average microbial air counts and diversities for containers followed by standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Unwashed	36	422 $\pm$ 308 a	1.0 $\pm$ 1.0 a	6 $\pm$ 6 b	1.3 $\pm$ 1.1 a
Washed	36	367 $\pm$ 334 a	1.1 $\pm$ 0.8 a	94 $\pm$ 139 a	1.2 $\pm$ 0.5 a
<i>F probability</i>		0.573	0.931	<0.001	0.389
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Unwashed	36	428 $\pm$ 307 a	2.3 $\pm$ 1.0 a		
Washed	36	460 $\pm$ 372 a	2.3 $\pm$ 0.9 a		
<i>F probability</i>		0.595	0.962		

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

APPENDIX U Average microbial air counts and diversities for cold storage facilities in South Africa followed by standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Farm 1	12	55 $\pm$ 166 a	2.7 $\pm$ 1.2 b	13 $\pm$ 7 b	2.0 $\pm$ 0.9 a
Cape Town	36	101 $\pm$ 210 a	1.0 $\pm$ 0.9 a	3 $\pm$ 2 a	1.4 $\pm$ 1.1 a
<i>F probability</i>		0.465	<0.001	<0.001	0.078
Farm 2	12	79 $\pm$ 113 a	1.8 $\pm$ 0.9 a	52 $\pm$ 20 b	1.3 $\pm$ 0.4 a
Cape Town	36	73 $\pm$ 152 a	1.0 $\pm$ 1.0 a	3 $\pm$ 2 a	1.4 $\pm$ 1.1 a
<i>F probability</i>		0.916	0.03	<0.001	0.775
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Farm 1	12	68 $\pm$ 163 a	4.8 $\pm$ 1.5 b		
Cape Town	36	103 $\pm$ 210 a	2.5 $\pm$ 1.5 a		
<i>F probability</i>		0.545	<0.001		
Farm 2	12	131 $\pm$ 128 a	3.1 $\pm$ 1.0 a		
Cape Town	36	76 $\pm$ 152 a	2.4 $\pm$ 1.5 a		
<i>F probability</i>		0.348	0.172		

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

APPENDIX V Average microbial air counts and diversities for cold storage facilities in Europe followed by standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Antwerp	18	824 $\pm$ 878 a	0.9 $\pm$ 0.3 a	5 $\pm$ 5 a	1.0 $\pm$ 0.7 a
Rotterdam	18	689 $\pm$ 907 a	1.0 $\pm$ 0.4 a	4 $\pm$ 6 a	0.9 $\pm$ 0.7 a
<i>F probability</i>		0.799	0.126	0.660	0.707
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Antwerp	18	829 $\pm$ 876 a	1.8 $\pm$ 0.8 a		
Rotterdam	18	693 $\pm$ 905 a	1.9 $\pm$ 0.9 a		
<i>F probability</i>		0.788	0.756		

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

APPENDIX W Average microbial air counts and diversities for repacking facilities followed by standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Repacking facility 1	24	222 $\pm$ 299 a	0.9 $\pm$ 0.4 a	9 $\pm$ 18 a	1.0 $\pm$ 0.8 a
Repacking facility 2	24	250 $\pm$ 303 a	1.0 $\pm$ 0.5 a	8 $\pm$ 15 a	0.9 $\pm$ 0.6 a
<i>F probability</i>		0.809	0.430	0.783	0.717
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Repacking facility 1	24	231 $\pm$ 307 a	1.9 $\pm$ 1.0 a		
Repacking facility 2	24	258 $\pm$ 307 a	1.9 $\pm$ 0.6 a		
<i>F probability</i>		0.815	0.919		

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

APPENDIX X Average microbial air counts and diversities for retail centres followed by standard deviation ($\pm$ SD)

		FUNGI AND YEASTS		BACTERIA	
Sampling area	n	Population density	Population diversity	Population density	Population diversity
Retail centre 1	12	359 $\pm$ 337 a	1.1 $\pm$ 1 a	13 $\pm$ 31 a	0.7 $\pm$ 1.0 a
Retail centre 2	12	259 $\pm$ 360 a	0.8 $\pm$ 0.4 a	3 $\pm$ 2 a	1.0 $\pm$ 1.2 a
<i>F probability</i>		0.680	0.481	0.030	0.446
TOTAL MICROORGANISMS (FUNGI, YEASTS AND BACTERIA)					
Sampling area	n	Population density	Population diversity		
Retail centre 1	24	373 $\pm$ 327 a	1.8 $\pm$ 1.2 a		
Retail centre 2	24	262 $\pm$ 360 a	1.9 $\pm$ 1.4 a		
<i>F probability</i>		0.627	0.808		

a,b Means per column followed by the same letter did not differ significantly at the 1% level.

APPENDIX Y Theoretical density of normalized colour and its development in time
(Tijskens *et al.*, 2003).

