The risk of pathogenic microbiological contamination of South African fresh fruit for the export and local market

By

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Declaration

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Abstract

In South Africa microbiological safety of fresh fruit is not verified independently, and microbiological safety of fruit depends on producers and implementers of food safety systems and food safety auditors. Research and documented outbreaks of food poison incidents indicates that pathogens such as *Salmonella spp., Escherichia coli* and *Listeria monocytogenes* are associated with the fruit production and packing environment. The mechanisms of contamination in these environments were well established, but the amount of fruit produced makes it difficult to determine risk by doing microbiological analysis on randomly drawn samples from export batches or consignments of fruit. This study investigated the microbiological safety of fresh fruit produced in South Africa.

Results from microbiological testing on samples drawn from fruit exported to Indonesia, which was the only export market requiring batch microbiological analysis, was used. 2688 Samples were analysed, including citrus fruit, pome fruit and table grapes for the presence of *Salmonella* and *E. coli*. Only 3 pear samples tested positive for *E. coli,* but still within acceptable levels.

This study also indicated that consumers need to be educated on food safety principles in selecting and consuming fresh fruit, as some fruit varieties proved to be at higher risk than others for example, melons were classified as high risk as opposed to citrus fruit which was of low risk. The study emphasized the importance of food safety programs in making sure that fresh fruit was produced and packed in environments where microbiological risks was managed. It also confirmed that environmental microbiological testing is the preferred tool in determining risk and prevention of microbiological contamination. A final important factor established was that implementers of food safety systems should have good knowledge of microbiological risks in their environments and be able to interpret microbiological analysis correctly to prevent contamination of fruit produced. Authorities evaluating the implementation of food safety systems should be equipped with suitable knowledge.

Key words: Fresh fruit, Pathogens, Food safety, Fruit production environment

Opsomming

In Suid Afrika word die mikrobiologiese veiligheid van vars vrugte nie deur ‘n onafhanklike regulerende instansie bepaal nie en die mikrobiologiese veiligheid van vrugte is afhanklik van produsente, die implimenteerders van voedselveiligheidsisteme en voedselveiligheids auditeurs. Navorsing en gedokumenteerde voedselvergiftigingsuitbrake dui daarop dat patogene soos *Salmonella spp., Escherichia coli* and *Listeria monocytogenes* dikwels geassosieer word met vrugte, vrugteproduksie en die pakhuis omgewing. Die meganismes van kontaminasie van vrugte en die produksie omgewing met hierdie patogene is bekend, maar die groot volumes vrugte wat geproduseer word maak dit moeilik om met mikrobiologiese toetse op monsters wat ewekansig uit hierdie groot volumes vrugte getrek word, presiese mikrobiologiese risiko of teenwoordigheid van patogene op uitvoer vragte vrugte te bepaal. Hierdie studie het die mikrobiologiese veiligheid van Suid Afrika se uitvoervrugte ondersoek.

Die resultate van mikrobiologiese ontledings op vrugte bestem vir die Indonesiese uitvoermark was gebruik in die studie aangesien die mark die enigste mark is wat vereis dat vrugte mikrobiologiese ontledings moet ondergaan. 2688 vrugtemonsters (sitrus, kernvrugte en tafel druiwe) wat getoets was vir die teenwoordigheid van *Salmonella* en *E. coli* se resultate was nagegaan. Uit hierdie monsters was daar net 3 monsters wat positief getoets het vir *E. coli*, alhowel dit nog heeltemal binne aanvarbare norme was.

Die studie het aangedui dat verbruiker opvoeding aangaande voedselveiligheids beginsels rondom vars produkte soos vrugte belangrik is aangesien die risiko van voedselvergiftiging gekoppel aan vrugte nie dieselfde is vir all vrugte nie en dat vrugte soos spanspek en avokados ‘n groter risiko het as bv. Sitrus vrugte en appels. Die studie het die belangrikheid van voedselveiligheids programme in die produksie sowel as pakhuis omgewing uitgelig sowel as die noodsaaklikheid van goeie voedselveilgheids opleiding en verantwoordelike produsente. Die studie het bevestig dat mikrobiologiese omgewings studies meer waarde het om potensieele bronne van mikrobiologiese kontaminasie te indentifiseer en dan te bestuur. Die laaste belangrike aspek wat uitgelig was, was dat die implimenteerders van voedselveiligheids sisteme besonder goeie kennis van die mikrobiologiese risiko’s veral in die produksie omgewing moet handhaaf en veral instaat moet wees om mikrobiologiese

resultate korrek te kan interpreteer om moontlike kontaminasie van vrugte te kan voorkom. Dit is ook belangrik dat instellings verantwoordelik vir die implemntering van voedselveiligheid in die vrugte produksie omgewing baie goed opgelei moet wees.

Sleutelwoorde: Vars vrugte, Patogene, voedselveiligheid, vrugte produksie omgewing

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Chapter 1: Introduction

1.1. Introduction

Over the last few years there has been an increase in cases of food poisoning associated with fresh produce, particularly fresh fruit. Questions that needs to be addressed includes, how does fresh fruit get contaminated with pathogens and which pathogens were of greatest concern? Do all the systems currently in place in the fresh fruit and vegetable supply chain prevent microbiological contamination? Can scientists accurately determine microbiological safety and eliminate or reduce contaminated fruit before it reaches the end consumer? The key question was whether the fresh fruit ending up on the end consumer's plate was safe to consume? To determine this, this study looked at the available Indonesian microbiological data for fresh fruit exported from South Africa to determine its safety. The study also looked at what was currently in place to ensure consumer safety in terms of microbiological analysis and other solutions that could be used to ensure microbiological safety. This study aims to investigates if microbiological risk should be addressed in with an independent microbiological sampling process.

Questions that would be investigated in this study to assist in resolving this problem include:

1. Could PPECB provide proof, independently, to the importing countries, that SA fresh fruit was safe in terms of pathogens? (Could the Indonesia microbiological databank with all microbiological analysis from 2014 onwards, be a benchmark to assess current microbiological status of SA fresh fruit?).

2. Was it possible to detect and eliminate fruit from being exported if microbiological contaminants/pathogens were present, a pro-active outcome?

3. Would it be possible to build a database of microbiological safety of exported fruit over time and develop an early warning system – determining the risk?

Additional questions that needs to be addressed in solving this issue (which was critical for consumers of fresh fruit locally as well):

4. Could consumers or the custodians of the export and local fresh produce markets really assume that the fresh fruit and vegetables supplied and consumed were

microbiologically save? (This is the current assumption, based on food safety audits done on primary production and packing facilities).

5. What micro-organism should be tested for, was it enough to look at *E. coli* and *Salmonella*, which was most likely post-harvest contaminants, what pathogens could contaminate fruit during the growing phase (especially from the soil and irrigation water)?

6. Can the authorities follow a similar SOP for microbiological contaminants as was currently being used to verify MRL (maximum residue level) compliance? The MRL SOP verify pesticide residue compliance for every producer at the beginning of packing of each product, this would determine time, frequency, production unit coverage and type of microorganisms (Independent audit samples).

1.2. Background

One of the key development goals of the United Nations (UN) is contained in Goal 2 which is to end hunger, achieve food security and improved nutrition and promote sustainable agriculture. The first step to achieve this goal was to end hunger by 2030 and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round (UN, 2019).

Food safety remains a critical factor in the food chain, even more so for fresh fruit and vegetables considered by nutritionists as an important element of a nutritious and balanced diets. A food safety failure in the fresh fruit production environment, apart from economic implications, could lead to consumer aversion and could also affect the sustainability of this sector.

According to the Standards and Requirements for all fresh fruit exported from South Africa to international markets, fruit must comply to the prescribed tolerance for Micro(biological) food safety hazards. No consignment of table grapes/ citrus/ pome/stone etc. classified as “Extra class”, “Class 1”, and “Class 2” shall contain biological or chemical contaminants in quantities or at levels that exceed the maximum limits prescribed in terms of the Foodstuff, Cosmetics and Disinfectants Act, 1972 (Act no 54 of 1972) Agricultural Product Standards Act, 1990 (Act No. 119 of 1990). The Foodstuff, Cosmetics and Disinfectants Act, 1972 (Act no 54 of 1972) also makes provision for the safety of all food including fresh fruit and vegetables

supplied to the South African local market. In this study, it was important to understand that the fresh fruit produced for export markets and local markets comes from the same production environments, with the top-quality fruit normally exported and the rest packed for local consumption. Therefore, they were utilizing the same pack houses subjected to the same monitoring conditions and food safety requirements and standards.

Although this microbiological requirement was stipulated, no produce (fresh fruit and vegetables) were being tested for microbiological contaminants (per the stipulated legislative requirements detailed above) by any of the independent regulatory bodies (DALRRD/PPECB) at the time of this study. Only one export destination, Indonesia, require microbiological analysis (Indonesian Ministerial Decree No. 234 of 2016). The Indonesian food safety requirement include heavy metals, MRL (maximum residue level on pesticides) and the microbiological testing include *Salmonella*, and *E. coli* (Indonesian Ministerial Decree No. 234 of 2016). This study aims to determine if this status quo should be maintained, what the impacts were on fruit quality and consumer health or if microbiological analyses should be done for all markets.

In the case of microbiological contaminants and the tolerances allowed for pathogenic micro-organisms, Act 54 currently refers to the Codex Alimentarius as a guideline for fresh fruit and vegetables. The codex according to the Commission Regulation (EC) No 2073/2005 of 15 November 2005 prescribes limits as follows:

- *Salmonella*: absent in 25g
- *Escherichia coli*: 100 cfu/g (In relation to this study, Indonesia has a stricter requirement of <20 cfu/g).
- *Listeria monocytogenes*: On ready to eat food that can support the growth of Listeria – absent in 25g and Listeria monocytogenes: On ready to eat food that cannot support the growth of Listeria – 100 cfu/g.

The sampling of fruit for microbiological testing, based on this requirement, was the function of the inspection body PPECB (Perishable Product Export Control Board) as an assignee of DALRRD (Department of Agriculture Land Reform and Rural Development, previously DAFF – Department of Agriculture Forestry and Fisheries). No microbiological testing on fresh fruit

and vegetables for the local market was done by any of the South African government agencies involved with agricultural produce.

In 2018 Egypt became the first export destination requiring all grains, fruit and vegetables from South Africa be tested for *Listeria* (DAFF communication to PPECB, 10 July 2018), due to the Listeriosis outbreak linked with cold meats (NICD, 2018). PPECB and DAFF anticipated, that export markets would follow suit in the medium term, and more extensive microbiological testing could therefore be expected. Since the Listeriosis outbreak in SA was resolved (NICD, 2018), Egypt withdrew this requirement and no additional requirements relating to microbiological testing has subsequently been requested by any other country. This prompted DAFF to abandon any projects relating to the microbial safety of fruit.

Currently, according to the Standards regarding food hygiene and food safety of regulated agricultural food products of plant origin intended for export, Notice 707 of 2005, all producers (farmers) and fruit pack houses must have an audited food safety system in place to be able to export fresh fruit from South Africa (Export conditions of consignments of regulated products from local/ National fresh produce markets, DAFF, 2014). Producers and pack houses wishing to ensure compliance to these systems such as GlobalG.A.P, BRC (British Retail Consortium) or HACCP (Hazard Analysis Critical Control Points), must test for pathogens such as *E. coli, Salmonella* and *Listeria*, on the products, employees hands, packing surfaces, and water used for washing products or cleaning and personal hygiene (SANNS 10330:2007; BRC Global standard for food safety, 2019).

It was an accepted industry practice that if a farm has a food safety system e.g. GlobalG.A.P (Good Agricultural Practices) and a pack house has a system e.g. HACCP or BRC in place and were audited against these food safety systems on an annual basis, the fruit and vegetables coming from these production sites and handling facilities were safe and compliant. PPECB employees working in fruit production sites and in pack houses, observe and report contradictory practices to that required in food safety systems such as GlobalG.A.P and HACCP. Food safety auditors audit these sites and facilities on an annual basis, visiting these sites for one day, allowing for covering up or window dressing of hygiene practices and activities on the farm.

In contrast to microbial risk the chemical residue risk on all fresh fruit and vegetables exported from South Africa is thoroughly tested by DALRRD and PPECB. This happens through the extensive and methodological drawing of samples for MRL testing of fruit and vegetables samples from all production units, including all fruit types and varieties, independently on an audit principle and sent in for analyses for MRL's (maximum residue levels) to accredited laboratories. The independent sampling eliminates producer bias and results in a robust system.

The export of fresh produce (agricultural value chain) plays a huge part in the South African economy and was identified as one of the commercial sectors that can lead to further job creation (Foodtradesa, 2019). As such South Africa has an obligation towards international and national food safety and security and is vulnerable to food poisoning outbreaks linked with South African fresh produce (Foodtradesa, 2019). This has direct bearing on producers and the South African economy directly affecting market access (Foodtradesa, 2019).

Of major concern was the fact that these results were never disclosed or published externally for scrutiny by interest groups. The results (Food safety certificates) from microbial safety testing for fruit exported to Indonesia was stored with PPECB since 2014.

1.3. Formulation of research topic, Problem, Intervention, Comparison and Outcomes (PICO).

Food poisoning epidemics linked to fresh fruit and vegetables increased over the last few years (Yeni et al, 2016; Wadamori et al., 2017; Alegbeleye et al., 2018 and Bartz et al., 2017). South Africa as an exporting country of fresh fruit and vegetables, must be aware and must put in place independent food safety measures to monitor and prevent occurrence of these microbial food safety risks. The main responsibility lies with the producers and pack houses to comply with the compulsory food safety systems, however ultimately DALRRD and assignees such as PPECB, which remains responsible for overseeing the quality and food safety of all fresh fruit exported from South Africa, must be able to prove independently to our export markets that SA can supply safe fresh produce. Should it become necessary DALRRD and PPECB cannot depend on producers or pack houses to supply reliable

microbiological information. Solutions would be independent microbiological sampling and analysis, such as the current system for Indonesia or the establishment of how effective and reliable producers and pack houses complies to and implement their food safety systems. The Indonesian results can possibly be used as a reflection on this.

During orchard inspections it was evident that many orchards, especially those close to human settlements, main roads and farm worker accommodation were at significant hygiene risk in terms of proximity to human activity. The question then becomes "could these unhygienic conditions translate to microbiological contamination of fruit" (especially pathogens including viruses).

Post-harvest treatment also needs to be taken into consideration. For example citrus fruits were washed and treated with fungicides and waxed (low risk), as opposed to products such as melons, berries, pome and stone fruit which were normally not washed while table grapes and strawberries, where no washing was allowed at all (High risk).

1.3.2. Potential interventions to address the microbiological risk linked with fresh fruit

Microbiological analysis of fruit samples (post packing), of exported fruit, for all pathogens commonly linked with fresh fruit and vegetables (collecting microbiological data), through all production areas or alternatives to manage the microbiological risk.

Establishing of critical limits (the number of pathogens that could be tolerated)

Validation of sampling methods (sampling would be critical in terms of preventing contamination by sampler and sampling methodology)

1.3.3. Comparison

Based on literature study microbiological data from this study:

Fruit and vegetable classification according to risk

Potential high-risk production areas or production practices could be identified (including water)

1.3.4. Outcomes expected for this study

Microbiological testing protocol for fresh fruit and vegetables exported from South Africa.

Sampling methodology sampling frequency, based on risk profile of the fruit and vegetables, production environment, and handling practices.

Chapter 2: Literature Review

2.1. Microbiological contamination risks associated with fresh fruit

Linking fruit to microbiological pathogen contamination and consequently to a food poison outbreak is complicated. Fresh fruits and vegetables are generally perceived to be safe, because of the health benefits ascribed to them (Sapers, et al., 2009; Callejo´n, et al., 2015; Yeni, et al., 2016; Alegbeleye, et al., 2018). Fresh fruit and vegetables are not historically linked to food poisoning outbreaks, since meat, dairy and other high protein foods were considered higher risk (Sapers et al., 2009; Callejo´n et al., 2015; Yeni et al., 2016; Alegbeleye, et al., 2018). Developing countries face the biggest challenge, gastrointestinal diseases are endemic, unsafe agricultural practices are common, and poor sanitation often linked to poor water quality or unsafe water. Illnesses caused by consumption of locally grown fresh produce are a common occurance (Alegbeleye et al., 2018). This is potentially true for most of the developing countries, but also to some extent for South Africa, depending on production area and producer (WHO, 2019; Alegbeleye et al., 2018).

Apart from linking an outbreak to specific fresh produce, the reporting process was further complicated by delays and variability in diagnostic testing procedures, reporting of results, and in conducting epidemiologic investigations (Bartz, et al., 2017). In addition to the difficulty in linking fresh fruit to an outbreak, perishable food such as fruit is linked to a short shelf-life, and possible sources of contamination, are not always available for laboratory testing when an outbreak was established (Sapers, et al., 2009; Callejo´n, et al., 2015 and Bartz et al., 2017). Traceability to the source is also complicated by poor record-keeping and mixing of fruit from different suppliers through the supply chain. Identifying the specific source of an outbreak at the farm or field level is therefore often not possible (Sapers, et al., 2009; Callejo´n, et al., 2015 and Bartz et al., 2017).

Another problem in associating microbiological outbreaks with fresh fruit is the sporadic nature of outbreaks, the linking pathogens on fresh fruit to localized contamination events makes a systematic study of sources of contamination very difficult (Sapers, et al., 2009; Callejo´n, et al., 2015 and Bartz et al., 2017). The best approach is to assess the magnitude of the problem by obtaining data on the prevalence of produce contamination for different

commodities and production locations (Sapers, et al., 2009, Bartz et al., 2017 and Yeni et al., 2016).

According to Food safety news (FSN), recent epidemics linked to pathogens from fresh fruit and vegetables include:

- 77 cases of illness, linked to *Salmonella* in pre-cut melons in the USA (FSN, 2019).
- *Salmonella* on cucumbers, 147 people confirmed sick in the EU (FSN, 2019).
- In 107 countries including the EU and USA *Listeria* was found in frozen vegetables from one manufacturer, in the EU alone 47 people was infected with 9 deaths, as reported on 19 July 2018 by FSN.
- In Sweden 13 people have been infected With Hepatitis A, linked to Frozen Strawberries (FSN, 2018).
- On 7 July 2018, 212 people were confirmed to be infected, in 4 states of the USA, with *Cyclospora* parasites linked to precut vegetables (FSN, 2019).
- By the 24th of May 2019, 157 confirmed cases of *Salmonella* poisoning by pre-cut melons were reported in the USA, these melons were distributed over ten states between April and May 2019 (FSN, 2019).
- On 22 May 2019, the USA FDA confirmed reports from Wisconsin and Minnesota about *Salmonella* infections connected to fresh vegetable trays from Del Monte Fresh Produce Inc (FSN, 2019).

In an intervention by the US Food and Drug Administration (FDA) to develop preventive controls against pathogens such as *hepatitis A, norovirus* and other bacterial pathogens, 2,000 samples of frozen berries produced in and/or imported to the United States were analyzed (FSN, 2019). These two viruses caused outbreaks in recent years which compromised the health of hundreds of people across the United States (FSN, 2019). Berries are served raw or frozen and is seldom subjected to further processing steps that can kill viruses and bacteria, increasing the risk of foodborne pathogens compared to other fresh or processed fruit (FSN, 2019). The preventative testing program for the USA berries started in November 2018 and will take 18 months to complete, illustrating timelines for investigations like this. A final analysis is planned after the data was reviewed. Berries are delicate and may become

contaminated with bacteria or viruses if handled by an infected worker who does not use appropriate hand hygiene, or if exposed to contaminated agricultural water or a contaminated surface during picking and packing (FSN, 2019, Sapers et al., 2009, Bartz et al., 2017 and Yeni et al., 2016). Freezing preserves berries but generally does not kill viruses and bacteria which can survive at low temperatures (FSN, 2019, Bartz et al., 2017 and Yeni et al., 2016)).

2.1.1 Incidents that was a concern for the South African fruit industry:

On 27 December 2017, Food safety news reported on the biggest recall in history, of fresh apples in the USA, due to *Listeria* contamination. At this point 7 people have already died, prompting research to start on *Listeria* linked to fresh fruit (FSN, 2019).

Due to the USA outbreak of Listeriosis linked to apples, South African apples were also under suspicion, since SA was already flagged due to the 2018 Listeriosis outbreak associated with cold meats. SA apples were consequently tested and proven to be safe (Hortgro, 2018).

On 9 April 2018, the WHO reported on a Listeriosis outbreak linked to rock melons from Australia, which had been exported to several countries. Several cases of infection as well as 7 deaths in Australia were reported. Effective recall procedures were subsequently implemented (WHO, 2018).

In one of the most recent *Listeria* connected food safety alerts, Henry Avocado Corporation in the USA voluntarily recalled its California-grown avocados sold in bulk at retail stores since the avocados had the potential to be contaminated with *Listeria monocytogenes* (FDA, 2019). The withdrawal was precautionary based on positive test results on environmental samples taken during a routine government inspection at its California packing facility however there were no reported illnesses associated with this recall (FDA, 2019).

The CDC (Centre for disease control) in South Africa linked the following food borne pathogens to fresh fruit and vegetables: *Cyclospora, Escherichia coli, Listeria, Norovirus* and *Salmonella* (CDC, 2019).

The NICD-NHLS handbook for diagnosis of foodborne illness clusters/ outbreaks, identified and have emergency protocols in the event of disease outbreaks in place for South Africa for the following organisms, as the main causes of food borne illness: *Salmonella spp., Shigella spp., E. coli O157*, *Bacillus cereus, Clostridium perfringens*, *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio* spp. (NICD, 2016).

The following organisms were most commonly linked to food borne illness associated with fresh fruit and vegetables: Various *Salmonella spp., E. coli 0157:H7*, *Listeria monocytogenes, Campylobacter jejuni*, *Norovirus, and Hepatitis A* (Yeni et al., 2016; Rajwar, et al., 2016; Wadamori, et al, 2017; Alegbeleye et al., 2018 and Zhu, et al., 2017).

Researchers noted that there were also an increased association between fresh fruit and food poisoning incidents. This could be attributed to an increase in the consumption of fruit and vegetables due to the associated health benefits. Improvements in cold chain and packaging also made it possible to import and export fruit to more markets, providing year-round supply of fruit and vegetables to more people (Alegbeleye et al, 2018; Bartz et al., 2017; Yeni et al., 2016 and Wadamori et al., 2017). Convenience could also be associated with an increased risk for contamination, as more and more people prefer to consume prepared and pre-cut vegetables and fruit (Yeni et al., 2016). The processing steps of peeling, cutting, mixing and packaging increase the risk of mechanical contamination (Wadamori et al., 2017). Another concern was the worldwide increase in water and soil pollution with pathogens such as *Salmonella* and *E. coli* (Heaton et al., 2008). Another recent concern specifically to *Salmonella*, was the possibility that future outbreaks will become more severe (Cliff, et al., 2019). Researchers in Australia linked this to the close degree of pathogen separation within *Salmonella* networks, which would lead to the emergence of increasingly aggressive strains of *Salmonella*, ultimately creating a superbug, as is the case with other human pathogens (FSN, 2019; Cliff et al., 2019).

The risk of pathogen outbreaks through consumption of fresh fruit and vegetables, minimally processed (skinned and sliced for convenience) and processed (for example fruit juice and frozen fruit or fruit pieces) were not only limited to the country of production but across

borders as well. This was due to better distribution and cold chain infrastructure (Sapers, et al, 2009; Alegbeleye et al, 2018; Bartz et al., 2017; Yeni et al., 2016 and Wadamori et al., 2017).

South Africa is currently exporting 2,7 million tons of fresh fruit and vegetables to more than 90 countries, this industry is worth R26 billion ($2,4 billion) and continues to grow annually (FPEF, 2019). Any food safety issue such as a microbiological disease outbreak could have a devastating effect on this industry and subsequently the South African economy (Foodtradesa, 2019).

The importance of the fresh fruit and vegetable industry both nationally and internasionally has led to an increased concern regarding the safety of all fresh fruits and vegetables that were consumed with or without processing. The investigation into the control or elimination of any microbiological hazard have therefore become a very important field of research (Sapers, et al., 2009; Callejo´n, et al., 2015; Alegbeleye et al., 2018; Yeni et al., 2016; Wadamori et al., 2017).

In the South African fruit export industry these concerns were mainly addressed by a food safety focus in terms of the standards regarding food hygiene and food safety of regulated agricultural food products of plant origin intended for export, Notice 707 of 2005, which requires that all producers and processors of fresh fruit and vegetables needs to obtain a Food safety certificate such as GlobalG.A.P on farms and HACCP (Hazard analysis critical control points) and BRC (British Retail consortium) certification for Off-Farm pack houses, before they were allowed to export. In principle the South African government through the Department of Agriculture Land Reform and Rural Development (DALRRD) relies only on this certification as a guarantee of food safety, with no additional requirements apart from systematic agricultural chemical residue testing regime.

Compared to food types such as meats, eggs, and dairy products the overall incidence of foodborne illness for fresh fruits and vegetables are relatively low and none of the usual human pathogens typically associated with causing foodborne illness such as *E. coli, Salmonella* and *Listeria monocytogenes* were considered endogenous microflora of fresh fruits, these pathogens will rather occur as contaminants (Sapers et al., 2009; Alegbeleye et al, 2018; Bartz et al., 2017; Yeni et al., 2016 and Wadamori et al., 2017).

Contamination happens during either the production (farm) or packing process (Sapers, et al., 2009; Wadamori et al., 2016; Alebeleye et al., 2018 and Bartz et al., 2017). Fresh fruit generally provides an environment hostile to the growth and survival of these pathogens, however despite the low incidence of fresh fruit containing these microbes, the occasions where there was an occurrence, the risk for the consumers were very high (Alegbeleye et al., 2018). Reasons for this include the fact that most fresh fruit were consumed as part of a healthy diet and in most cases fresh fruit were consumed raw without any preparation or minimal preparation. Many people do not wash fresh fruit before consuming or preparing it for consumption, for example peeling my transfer microbes on the surface to the edible part (Sapers, et al., 2009; Alegbeleye et al., 2018).

From the literature it is possible to conclude that many foodborne illnesses caused by fresh fruit and vegetables were not even traced back to fresh produce consumption due to the associated health benefits and consumer ignorance (Bartz et al., 2017 and Rajwar et al., 2016). Consequently, if fresh fruit or vegetables were positively linked to food borne illness, fresh fruit could be viewed more negatively by the consumer, which will impact future consumption as well as the fresh produce and export industries. This would stand in contrast to an outbreak associated with a food that was more readily suspected such as cooked or processed meat, dairy and egg products, which has a limited impact on consumption, as consumers expect this to happen from time to time.

Fresh fruit and vegetables provide circumstances where pathogenic microorganisms are very unlikely to grow or multiply, however it remains important to investigate these pathogens especially the ones with low infectious dose but also pathogens that would have the capability for survival post-harvest and through the distribution chain (Sapers, et al., 2009 and Alegbeleye et al., 2018).

When considering fresh fruit as a source of foodborne disease the three most common microbes to consider are *E. coli* (of which *E. coli O157:H7* was the most prevalent strain), *Salmonella*, and *Listeria monocytogenes* (Yeni et al., 2016; Rajwar et al., 2016; Wadamori et al., 2016; Zhu et al., 2017 and Alegbeleye et al., 2018). The fact that *Salmonella* and *E. coli* were occasionally associated with fresh fruits could be attributed to their tolerance to

extreme conditions (Sapers et al., and Bartz et al., 2017). *Salmonella species* are resistant to desiccation, which aids in its survival on the surface of fresh fruits (Sapers et al., 2009; Bartz et al., 2017). *Salmonella* and *E. coli* are resistant to acids. Acid tolerance is based on the adaptability of these microbes in stationary phase. For this reason, both *E. coli* and *Salmonella species* are observed to survive in the relatively acidic fresh fruit environment (Sapers, et al., 2009; AFDO, 2009 and Yeni et al., 2016). *Listeria monocytogenes* is of concern when it comes to fresh fruits, especially where these fruits were going to be peeled or cut during preparation for eating (Zhu et al., 2017 and Yeni et al., 2016). Another big concern is fruits and vegetables produced close to soil and with an uneven rough skin. Examples such as melons and avocados recently dominated food safety linked to *listeria*, as with *Salmonella* and *E. coli, Listeria* is also capable of growing under extreme conditions as well as the acidic environment associated with fresh fruit (Yeni et al., 2016; Rajwar et al., 2017 and Zhu et al., 2017) *Listeria* is an ambiguous bacteria in the fruit production environment and was frequently found on fruits and vegetables in all production areas investigated (Sapers, et al., 2009, AFDO, 2009 and Yeni et al., 2016).

It is possible to group fresh fruit into different risk groups, ranging from low to high, based on production and harvesting practises. The ability of the pathogen to internalise into the fruit, as well as post-harvest treatments (Sapers et al., 2009 and Alegbeleye et al., 2018). Applying this concept, apples were considered high risk because of the well documented internalization of pathogens whereas citrus fruit was considered low risk because of the very low possibility of internalisation of pathogens as well as the relative thick skin and various post-harvest treatments including washing and waxing. Melons on the other hand were also classified into a very high-risk category due its surface characteristics and production close to soil (Sapers, et al., 2009 and Alegbeleye et al., 2018).

When looking at the risk associated with eating fresh fruit it is important to consider that a single contaminated fruit will likely result in illness of only a single consumer or a few consumers spread over the whole distribution area of contaminated fruit from one producer, consequently, detection or characterisation of outbreaks due to individual contaminated fruit may be very difficult, resulting in underreporting or even missing a food poisoning outbreak related to fresh fruit (Alegbeleye et al., 2018 and Kumar et al., 2018). As mentioned,

consumers tend to not suspect fruit as a carrier of pathogens. This problem becomes much bigger if fruit was peeled and sliced or diced into fresh fruit salads and even more when processed into fruit juices (Sapers et al, 2009 and Li, et al., 2018). Outbreaks are more often attributed to fruit salads, especially if melons formed part of the ingredients and or multiple pieces of fruit were mixed. As a result, it is not possible to pinpoint the exact ingredient in a fruit salad responsible for the initial contamination illustrating the importance of understanding the risk associated with fresh fruit as carrier of pathogenic bacteria, particularly during fresh consumption, preparation and processing (Li et al., 2018). It is important to understand that fresh fruits and vegetables can become contaminated at any point during production, picking, packing and/or processing and result in foodborne illness (Sapers, et al., 2009; Bartz et al., 2017; Alegbeley et al., 2018 and Li et al., 2018). The Indonesia set of microbiological results might give a good indication on South Africa's prevalence to possible microbiological contamination.

2.2. Sources and routes of microbiological contamination of fresh fruit

Foodborne pathogens such as *Salmonella, E. coli* and *Listeria* are not typically considered to be part of the normal surface micro-organism populations of fresh fruits because of factors such as low water activity, acidity or protection provided by the fruit surface environment itself (Alegbeleye et al, 2018; Kumar et al., 2018 and Bartz et al., 2017). Recent research suggests that the surface environment of fresh fruit can provide adequate and favourable conditions for growth and/or survival of foodborne pathogens (Alegbeleye et al, 2018; Kumar et al., 2018; Bartz et al., 2017 and Wadamori et al., 2017). For example, pathogens such as *Listeria monocytogenes* can survive in a biofilm firmly attached to the surface of the fruit. In other fruits for example pome fruit (apples and pears), growth and or survival may occur internally, in the fruit flesh, especially where the fruit surface was damaged or injured. Once pathogens such as *Salmonella, E. coli* and *Listeria* were introduced to the fruit surface or flesh, the pathogens are not easily removed (Sapers, et al., 2009; AFDO, 2009; UFPA, 2010; Zhu et al., 2017; Alegbeleye et al., 2018 and Rajwar et al., 2016).

Listeria monocytogenes is a pathogen associated with soil and was found to be prevalent in most fruit and vegetable production environments. The pathway to contamination of fruit during production and harvesting is therefore relatively easy (Zhu et al., 2017 and Yeni et al., 2016). *Salmonella* and especially *E. coli* is mostly associated with animal and human intestinal tracts. Human and animal activity can therefore introduce these pathogens into water sources used for irrigation, or become airborne together with dust particles and find its way through these mechanisms onto fruit (Sapers, et al., 2009; WHO, 2019; Rajwar et al., 2016, Kumar et al., 2018; Bartz et al., 2017 and Alegbeleye et al., 2018).

In recent years considerable effort was afforded into implementing food safety systems throughout the entire fresh fruit and vegetable production chain, both internationally and in South Africa (WHO, 2019). Referred to as Good agricultural practices (GAP) systems, such as GlobalG.A.P, which remains a prerequisite to export fruit from SA to most markets (Notice 707, 2005). GAP systems require that all activities on the farm or production sites must be evaluated against associated risks and would include analysis of the source of irrigation water, irrigation method and water quality (especially microbiological load in terms of pathogens). Water and the use of water during production, packing and processing remains one of the key factors in fruit contamination, consideration must therefore be given to the use of water on the farm such as for mixing plant protection products or fertilizers (Alegbeleye et al., 2018; Rajwar et al., 2016 and Bartz et al., 2017).

Another risk factor pertinent to microbiological contamination of fresh produce is soil. Soil or growth mediums and the use of compost or manure, which can also carry a substantial risk, to introduce pathogens must be considered and included in microbiological analyses (Alegbeleye et al., 2018; Rajwar et al., 2016; Bartz et al., 2017 and Sapers et al., 2009).

The most significant risk of introducing pathogens onto fresh fruit and vegetables during production, and during harvesting, was identified as human behavior. Hygiene training was identified as of the utmost importance (Rajwar et al., 2016; Bartz et al., 2017 and Sapers et al., 2009). As most fruit is picked or harvested by hand, farm workers need to be thoroughly trained on routes of microbiological contamination which is not only limited to personal hygiene but also protective clothing, picking equipment like scissors, picking bags, crates, and

transport equipment (Sapers et al., 2009; AFDO, 2009 and Bartz et al. 2017). Farm facilities such as toilets, potable hand wash water and sterilizing soap and chemicals for cleaning of equipment should be provided (WHO, 2019, AFDO, 2009). Farm worker behavior during the growth cycle of the fruit and during harvesting should be carefully monitored for implementation of good hygiene practices as well as behavior during harvesting in line with the specific risk associated with the fruit type (Sapers et al., 2009). A different approach can be followed with a low risk product such as citrus versus a high-risk product such as strawberries. Issues such as post-harvest treatments and in field packing, also determines the associated risk (Sapers et al., 2009). Harvesting and processing are already well established in literature as playing significant role when it comes to fruit contamination and in various outbreaks linked to *Salmonella*, *E. coli* and *Listeria.* A positive link was found between soil samples as well as harvesting equipment, which significantly increased if it rained during the harvesting window (AFDO, 2009, Bartz et al., and Alegbeleye et al., 2018). Other routes of contamination of fruit before or during picking include the quality of the irrigation water, the quality of the water used to make up spray mixture. It is well documented that pathogens can survive despite pesticides contained in a mixture, picking up fallen fruit from the orchard floor and mixing it in with fruit picked from the tree and the presence of cattle, and other domesticated or wild animals in orchards (Sapers, et al., 2009; UFPA, 2010; Bartz et al., 2017; Alegbeleye et al., 2018 and Rajwar et al., 2016).

Research clearly indicate that food safety compliance depends on the farmer or producer and their understanding of food safety and how effectively they adhere to food safety measures (Sapers, et al., 2009; UFPA, 2010; Bartz et al., 2017; Alegbeleye et al., 2018 and Rajwar et al., 2016). A problem, specifically in the SA food safety context, is that the implementation of these food safety measures are only measured annually by a food safety auditor, spending only a few hours on a production site. The relative short time that is spend doing the annual food safety audit creates room for short cuts and hiding of poor hygiene practices. Another concern in the SA food safety context is that the producer was responsible for drawing all samples critical to the determination of food safety. For example, water samples of irrigation and other water used on the farm, soil samples, microbiological samples of the compost and other material used on the farm and fruit sent in for microbiological analysis. The only independent sampling in SA is the fruit samples drawn for microbiological analysis for fruit

destined for Indonesia by the Perishable Export Control Board (PPECB) as assignee of DALRRD formerly DAFF (Department of Agriculture Forestry and Fisheries). The lack of independent sampling was previously pointed out by GlobalG.A.P but up to now not addressed by DAFF. Audit quality was also dependent on the quality of the training of auditors and how thoroughly they do the audit inspection and how well they are monitored and evaluated in performing their food safety audit functions. The microbiological aspects of food safety in SA, especially on export fruit and vegetables are only covered by that of a current food safety certificate that must be in place for each production unit to be able to export from SA. This situation might eventually open the possibility of a foodborne illness transferred by fresh fruit from SA, and therefore the importance of this study.

Contamination of fresh fruit with pathogens would only represent an actual food safety hazard when pathogens in the production and pack house environment attached onto fruit surfaces, survive the environmental stresses including post harvest treatments, sanitizing agents. The pathogens must then multiply to a population level sufficiently to cause illness (Sapers, et al., 2009; AFDO, 2009; Rajwar et al., 2016; Alegbeleye et al., 2018; Bartz et al., 2017; Wadamori et al., 2017; Yeni et al., 2016 and Zhu et al., 2017). These questions can only be answered by research studies which can assist in the identification of the most effective methods of detection, as well as food safety interventions at farm and pack house or further processing activities (Sapers, et al., 2009; AFDO, 2009; Rajwar et al., 2016; Alegbeleye et al., 2018; Bartz et al., 2017; Wadamori et al., 2017; Yeni et al., 2016 and Zhu et al., 2017).

Researchers has shown that despite the implementation of strict food safety principles (GAP's) reports of the presence and even outbreaks of foodborne illness continue (Sapers, et al., 2009, UFPA, 2010; Rajwar et al., 2016; Alegbeleye et al., 2018; Bartz et al., 2017; Wadamori et al., 2017; Yeni et al., 2016 and Zhu et al., 2017).

Fruit contamination with pathogens are not only linked to efficient hygiene control and good agricultural practices, but also with post-harvest handling in the pack house. Fresh fruit and vegetables normally go through a washing process to remove dust and debris as well as through several hands during sorting and packing. Researchers have shown that that poor hygiene and sanitation during post-harvest processes could also led to microbiological

contamination of fresh fruit and vegetables (Bartz et al., 2017; Rajwar et al., 2016 and Alegbeleye et al., 2018). The most critical aspect in post-harvest handling of fresh produce was indicated as water quality. Water plays a role in various pack house operations such as transport, product washing processes, facility and personnel hygiene, cleaning and sanitation. The original water source should therefore be potable, and additional measures such as the addition of chlorine could be implemented (López-Gálvez, et al., 2019; Sapers et al., 2009 and Chen et al., 2016). Water should be changed regularly to prevent microbiological build up with continued use (Sapers et al.; 2009 and Chen et al., 2016). Researchers indicated that a high risk of internalization of *Salmonella* and other pathogens are possible, especially if these pathogens are already present on the fruit or in the water. The risk of contamination further increased with the use of a warm water bath followed by a cold-water rinse on fruits such as mangoes, apples, citrus and tomatoes (Sapers et al., 2009; AFDO, 2009 and Alegbeleye et al. 2018). When water is used as part of post-harvest processing it is important to note that buildup of microbes and nutrients which may sustain these microbes may be present (Sapers, et al., 2009; Alegbeye et al., 2018 and Chen et al., 2016). In addition to the pathogen risk associated with water the other significant risk was human contact and the hygienic state of packing lines and equipment such as spray nozzles, scrubbers, sizing equipment, conveyer belts and the pack house environment (Sapers, et al., 2009; Rajwar et al., 2016 and Bartz et al., 2017).

To mitigate the contamination risk in the pack house, it is a requirement to implement a “Hazard analysis critical control point (HACCP)” food safety system. This system is based on a thorough investigation of all processes involved during the handling of fresh produce in a pack house that can create a food safety risk, albeit chemical, physical or biological (microbiological) interventions should then be put in place to prevent these from occurring or warrant actions if it does occur (SANS 10330:2007). In combination with the HACCP plan there are normally pre-requisite programs (PRPs) (SANS 10049:2011). The PRPs deal with issues such as proper cleaning and sanitation, water quality, personal hygiene training and traceability to name but a few (SANS 10049:2011). The highest risk of contaminating fresh fruit with pathogens in the post-harvest handling of fruit reside in the water used to wash the fruit, as well as the quality of the water used to wash pack house surfaces and equipment. Of equal importance was the water used for personal hygiene. The other critical issue was

personal hygiene and the extent to which pack house workers understand the concept of personal hygiene (UFPA, 2010 and Bartz et al., 2017).

2.3. Post-Harvest methods to reverse microbiological contamination on fruit to reduce risk

Research has indicated that once fresh fruit and produce are contaminated with foodborne pathogens, it becomes extremely difficult to decontaminate and preserve it. Pathogens would hamper the effort to maintain the fruit quality and ultimately consumability (Sapers et al., 2009; Li et al., 2018). Options are limited to various surface treatments and these treatments typically vary in effectivity depending on the produce type, method of inoculation, level of inoculation, and method of pathogen recovery (Sapers et al., 2009 and Joshi, et al., 2013). As a rule, internally occurring pathogens cannot be removed (Alegbeleye et al., 2018 and FDA, 2019). In addition, pathogens such as Listeria may also exist in a biofilm or other protective state rendering surface decontamination methods ineffective, and treatment methods with greater penetration must be considered to destroy these hard to reach pathogens (Zhu et al., 2017). Irradiation of fresh fruit is an option which show potential (Sapers, et al., 2009).

An important aspect to take into consideration when it comes to the safety of fruit is to classify it according to risk, especially in fruit such as citrus fruit where internalization of pathogens was unlikely to occur (Li et al., 2018) Typically low risk fruit, such as citrus have less stringent HACCP requirements. When considering post-harvest safety and efficacy of the process to reduce pathogenic load it is important to minimize initial microbial loads (orchard environment and hygiene practices during harvesting) (Bartz et al., 2017 and Alegbeleye et al., 2018). Cleaning processes applied to the surface, post-harvest, must therefore be applied to clean fruit to start off with, without visible blemishes or damage. Damage to the protective peel could allow pathogens to internalize, rendering processing treatments ineffective. Pre-sorting of damaged and severely blemish fruit will also be a critical step in making fresh fruit safer to eat (Sapers, et al., 2009; Josi et al., 2013 and FDA, 2019).

2.4. Best practices in terms of microbiological determination of food safety

The ultimate responsibility towards preventing pathogen outbreaks inside the country as well as making sure fresh fruit and vegetables were safe for export lies with the South African Government. In South Africa, the Department of Agriculture, Land reform and Rural Development (DALRRD) as well as the Department of Health are the responsible entity and these departments should have measures in place to prevent, detect or investigate foodborne outbreaks and illnesses. To achieve this, these role players need expertise in the field of microbiological investigation including knowledge and experience in surveillance systems, epidemiological investigations, laboratory methods, bacterial and viral ecology, water engineering, and environmental investigations (AFDO, 2009). In SA the ability to put together task teams to investigate outbreaks and draw up action plans are readily available, this can be considered a re-active approach, but in terms of pro-active measures such as environmental scans to detect possible pathogens and outbreaks in advance were nonexistent. In terms of pro-active interventions DALRRD currently does not have adequate personnel with relevant knowledge and experience in this field.

Food safety is an integral part of the production of all fresh foods and the shared responsibility of all segments of the supply chain. Currently in the light of many documented outbreaks associated with pathogens on fresh fruit and vegetables, there are an increased awareness for the need to evaluate the food safety practices in the production of agricultural products. Consumer demand includes important requirements namely availability, convenience and food safety (Li et al., 2018). The use of a microbiological testing program is an important tool in assuring food safety (Wadamori et al., 2017). Internationally many standardized, documented, and practiced procedures for preventative measures and performance standards for the entire foodborne outbreak investigation process are available (Sapers, et al., 2009; AFDO, 2009; UFPA, 2010, FDA, 2019 and NICD, 2016).

It was important to understand that microbiological testing is not a guarantee of product safety, it was merely a tool to determine the microbiological status of fresh fruit products at one point in the food chain and should be part of an overall food safety system (FDA, 2019). Microbiological testing provides important information about an environment, a process, and even a specific product lot, but it is important that sampling plans and methodology are

properly designed and performed (Bartz et al., 2017 and Rajwar et al., 2016). Microbiological testing is a powerful tool, however when microbiological tests are not properly designed and performed, testing can provide inaccurate information that can easily be taken out of context and create unwarranted concerns or false reassurances about the safety of the product (Li et al., 2018). Before microbiological testing could be initiated, prerequisite programs must be in place (Bartz et al., 2017 and FDA, 2019). These should include programs that are appropriate to the specific crop or production environment, such as: Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Sanitation Practices, Hazard Analysis Critical Control Point (HACCP), Traceability and Recall Management (UFPA, 2010).

Microbiological testing can never determine whether fresh produce will be pathogen-free, unless 100% of all the fruit packed was tested. At best researchers or quality managers can get a result that no pathogen was detected and to interpret that correctly they need to understand the levels of sensitivity and confidence provided by the sampling plans and testing methodologies used (Bartz et al., 2107). The Codex, European Commission, International Commission on Microbiological Specifications for Foods (ICMSF) and the FDA recommended precautionary testing only when there was good evidence that there was a microbiological problem and that testing would help to identify and control the problem (FAO, 2019; EC, 2019 and FDA, 2019). It is important to understand that microbiological testing offers limited solutions and provides only a small glimpse of many possible scenarios, incorrect application of microbiological tests would waste resources (time and money as microbiological testing was very expensive and time consuming), and potentially could create a worse food safety situation or consumer reaction than if no testing was performed (UFPA, 2010). Rival food companies often make use misinterpreted or out of context microbiologic results to gain advantage over competitors, especially on social media platforms where the consumer could be influenced.

Microbiological testing programs should be science-based and driven by usable outcomes and could only be implemented if the reason for testing was clearly defined as food and consumer safety and there should be a relative certainty of detecting or preventing a food safety issue. This knowledge would allow one to identify the type of samples that must be collected, the sampling plan to be used, the specific test to be performed, and actions to be taken prior to

and after the test results were obtained (UFPA, 2010 and FDA, 2019). Typical reasons for testing in the fresh produce industry were identified as: meeting product specifications at consumption, baseline development and identification of risk factors, process capability/validation, process verification, investigative testing and remedial activity verification, and verifying that regulatory guidelines have been met (UFPA, 2010).

2.5. Types of microbiological testing methods

2.5.1. Routine or Product specification testing: Consignment (lot-by-lot), assess safety of lots, or batches of products in process.

The most common reason for microbiological testing in the fresh produce industry today was to comply with a product specification (UFPA, 2010; Van Schothorst, Zwietering, Ross and Buchanan, 2008 and Cordier, 2018). Inherent in any product specification were assumptions that the sampling and test methods will provide a standard deviation and level of confidence in test results such that the user could determine whether their specification was met (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018). Specifications should include: the product that must be tested, the test frequency, sample size and sample drawing principles (where, how much and how), target organism, test method, criteria (maximum limit allowed) and what actions must be taken should the limits be exceeded (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018) . An important criterion was that the product must remain in the supplier's control until the results clear the product for shipping (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018).

2.5.2. Verification or baseline determination and identification of risk factors.

Verification or baseline determination and identification of risk factors was normally only done occasionally to measure continued effectiveness of controls (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018). Examples included: environmental studies which assessed effectiveness of the GAP and GHP program and potential for cross contamination, investigational analyses in response to failure or deviation specifically to identify the root

cause and shelf-life, to validate the shelf-life and impact of factors affecting it mostly to profile microbiological changes occurring in product during the shelf-life of individual lots (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018).

Before using microbiological testing to assess quality, safety or process verification, it was important to understand what's statistically "normal". Microbiological testing could be useful to understand the range of microbial populations present and their preference to specific types of produce, the influence of growing and handling practices, season, weather, geography, environmental controls and production actions that may influence the microbial population presence (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018). Baseline assessment should take place over a long period of time to record the variability. Key elements of a baseline assessment are: standardization of test methodology to enable comparing and compiling of data, establishing the frequency, number of tests and/or period required to have confidence in the accuracy of the baseline, managing such data statistically and analysing this data for trends and patterns (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018).

2.5.3. Process capability or validation microbiological testing.

Process capability or validation microbiological testing was used to "validate" the process' capability to reduce a particular or overall microbial population, or at least to ensure that the process does not allow microorganisms to grow or spread throughout a specific lot (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018).

For validation tests it is important to understand the background and variability of microflora that comes with the test lot (levels and type). Samples were collected at specific points in the process, to determine the impact of individual steps (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018). Validation required an initial, fixed, predetermined number of repetitions and tests. Validation microbiologic tests are used to conclude whether under certain conditions processes are consistently capable of producing product with an acceptable level of microbial quality (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018). Benefits for the validated process were indicated as: an understanding of factors that were critical to control and to produce reliable results, limits at which those factors should be maintained,

and routine monitoring of the microbiological quality of individual lots could be greatly reduced (UFPA, 2010; Van Schothorst et al., 2008 and Cordier, 2018).

2.5.4. Process verification as a microbiological test method.

Process verification as a microbiological test method was used to verify or confirm that the production process step performs as anticipated (UFPA, 2010; Van Schothorsta et al., 2008 and Cordier, 2018). Process verification involves periodic, ongoing testing. Process validation was intended to demonstrate that the validation process was functioning as designed, i.e., researchers were not getting statistically non-significant results than those observed during the validation trials (UFPA, 2010; Van Schothorsta et al., 2008; Cordier, 2018 and Zwieteringa et al., 2016).

2.5.5. Microbiological investigative testing and remedial action verification.

Microbiological investigative testing and remedial action verification is used as a very effective tool to investigate sources and causes of unexpected microbiological results (UFPA, 2010; Van Schothorsta et al., 2008 and Cordier, 2018). In cases where a process verification test indicates a much higher than normal presence of one microorganism or an unexpected microorganism was detected in a finished product, targeted microbiological testing was used to investigate the source of the unexpected microorganisms or to verify that remedial action was successful in eliminating the contamination source (UFPA, 2010; Van Schothorsta et al., 2008; Cordier, 2018 and Zwieteringa et al., 2016).

2.5.6. Compliance or verification microbiological tests.

Compliance or verification microbiological tests was used to determine if regulatory guidelines are met and is used to demonstrate compliance to published regulatory guidelines or requirements (UFPA, 2010 and Zwieteringa et al., 2016).

2.5.7 Conclusions on microbiological testing

Key elements in the process of detecting micro-organism contamination of fresh fruit, in the South African context, lies in suitable sampling methods, independent and trained samplers, accredited laboratories with accreditation to perform the required tests, reproducible fast testing methods that were accurate and consistent, whilst being affordable. Laboratories should also be able to handle large volumes of tests during peak fruit harvesting periods (UFPA, 2010). The reality for the SA Fruit industry was the difficulty to test all batches of fruit for microbiological contamination although the SA Fruit export industry manage successfully to test for MRL's on every producer's first lot of each cultivar. The conclusion was that microbiological contamination is easier to prevent (GAP and HACCP) and that a better solution was environmental scans (both the production and packing environment).

In SA two production scenarios in fruit production is found, (i) producers who produce and pack their own fruit in on farm pack houses, and (ii) cooperative pack houses packing for many producers. The best quality fruit was packed for the export markets, and the rest of the fruit was sold on the local (SA) market. Substandard fruit was normally sold to juice extraction processors and other fruit processing factories. Most production units (Farms) in SA comply to the GlobalG.A.P good agricultural practices standard to be able to export from SA. Off-Farm pack houses, mostly co-operative pack houses, have HACCP or BRC certification in place to be able to form part of the export chain from SA.

2.6. Fruit contamination risk looking at specific products in relation to specific Pathogens (*E. coli, Salmonella* and *L. monocytogenes*)

Human pathogens that meet fresh fruit in the crop production environment can rapidly attach and strongly adhere to the fruit surface. This can occur to varying degrees depending on the properties of the fruit, such as smooth or rough skin surfaces, pH, water activity, humidity, temperature and contact time. Some pathogens, such as *Listeria* and *Salmonella* can also form biofilms on plant surfaces (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017 and Rajwar et al., 2016). The extent to which pathogens such as *Salmonella, Listeria* and *E. coli* could attach, survive and multiply on fresh fruit surfaces pre and post-harvest also dependents on the type and characteristics of the pathogen and the degree of

protection from environmental stresses provided by the microbial attachment site, nutrient availability, interactions with surface, other plant microbes and surface environment in terms of acidity (pH) (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017 and Rajwar et al., 2016).

Pathogen survival and growth is greater in porous or broken tissue (injuries) than on smooth tissue (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017 and Rajwar et al., 2016). Another important factor was that pathogens can also become internalized within plant tissues via attachment and infiltration at pores and injuries (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017; FDA, 2019 and Rajwar et al., 2016). Time interval between inoculation and washing during pack house treatments, is also important the longer the time lapse the higher the possibility of survival or the more difficult it was to reduce pathogen levels, microbial internalization, and/or biofilm formation was also more substantial the longer the time between contamination, washing and cold chain intervention (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017; FDA, 2019 and Rajwar et al., 2016).

Based on evidence in literature most produce-associated food poison outbreaks originate from primary production (on the farm) (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017; FDA, 2019 and Rajwar et al., 2016). But contamination can occur at any point in the food chain (Sapers, et al., 2009; Bartz et al., 2017; Alegbeleye et al., 2018; Zhu et al., 2017; Li et al., 2018 and Rajwar et al., 2016). Pathogens can be divided in two main groups: those most commonly associated with animals, namely *E. coli O157:H7* and *Salmonella* and those associated with humans such as *Cyclospora, Shigella* and *hepatitis A* (Sapers, et al., 2009; Yeni et al., 2016 and Bartz et al., 2018). *Listeria* were identified as an ever-present pathogen in the agricultural production environment, especially in the soil and represents a separate group (Zhu et al., 2016). In terms of these pathogen groups it is important to understand what remedial effect further post-harvest, pack house and storage actions (cold chain) would have in cases where fruit was contaminated before the farm gate (Rajwar et al., 2016, Li et al., 2018 and Wadamori et al., 2017).

2.6.1. The risk associated with berries

Berry production especially that of grapes and blueberries drastically increased over the last few years most notably in blueberries experiencing a tremendous increase in SA.

In Recent years food safety concerns were raised and food related illness linked with blackberries, raspberries, blueberries, and strawberries (Macori, et al., 2018 and Tavoschi, et al., 2015). The biggest concern relating to berry consumption was the fact that berries, including grapes, were often consumed raw and unwashed, which limits the removal of potential pathogenic microorganisms (Sapers et al., 2009; Tavoschi et al., 2015 and Macori et al., 2018). As with all other fresh fruit the most important preventative step was to minimise initial contamination by implementing Good Agricultural Practices (Sapers et al., 2009; Tavoschi et al., 20159 and Macori et al., 2018). Critical considerations with berries was the use of good quality compost, irrigation water of high quality, especially when considering berries such as strawberries and blueberries which grow relatively close to the ground, as well as clean and sanitized harvesting equipment and transportation vehicles (Sapers et al., 2009; Tavosschi et al., 2015 and Macori et al., 2018). Worker hygiene in berry production was another critical aspect, when handling the variety of berries as a commodity (Sapers et al., 2009; Tavoschi et al., 2015 and Macori et al., 2018). Maintaining a proper cold chain throughout delivery to the final customer was a further critical aspect, considering the relative short shelf life associated with berries (Concha-Meyer et al., 2014 and Zhou et al., 2018). There are many documented outbreaks associated with the different berry types and it was very possible that numerous outbreaks, caused by fresh berries from the berry group have gone undetected or unconfirmed mainly because berries were associated with tremendous health benefits and not commonly associated with food related illness (Concha-Meyer et al., 2014; Tavoschi et al., 2015 and Macori et al., 2018). Berries are involved in outbreaks due to contamination by farm and pack house workers, use of unsafe agricultural practices – especially water, and the fact that the export and import of berries continue to increase in importance as consumers want year -long availability of this product due to the associated health benefits (Sapers et al., 2009; Tavoschi et al., 2015 and Macori et al., 2018 and Zhou et al., 2018). Berries are characterized as highly perishable and require minimal handling and contact with water (Sapers et al., 2009; Tavoschi et al., 2015; Macori et al., 2018 and Zhou et al., 2018). Berries are not allowed to be washed or to meet with water during any stage of

harvesting or packing as berries were highly susceptible to mould growth (Sapers et al., 2009; Macori et al., 2018 and Zhou et al., 2018). To achieve this, sorting and packing often takes place in the field, a common practice in SA with grapes as well as blueberries, increasing the importance of good hygiene during production and harvesting, to mitigate the risk especially with grapes most pre-pack containers and even bulk containers does give the instruction, to wash the product directly before consumption.

Most of the food borne illness outbreaks associated with berries involved viruses such as the *hepatitis* A virus and *norovirus* and protozoan parasite especially *Cyclospora*, however berries have been associated with bacterial pathogens. For example, strawberries were positively linked to *Staphylococcus aureus, Salmonella* and *E. coli*, strawberries and blueberries were positively linked to *E. coli*, and grapes were linked to *E. coli* and *Salmonella* (Saper, et al., 2009; Tavoschi et al., 2015; Zhou et al., 2018 and FSN, 2019). With berries it is especially hard to determine outbreak history and patterns as well as original source of contamination. Outbreaks were very sporadic and unpredictable and trace-back seldom reaches the production site due to the short shelf life and the fact that berries were often consumed together with other fruit types in salads (Li et al., 2018). This provides a large possibility that the berries were cross-contaminated post-harvest by poor hygiene at the human interface or packing or processing equipment and surfaces (Sapers et al., 2009 Tavoschi et al., 2015 and Li et al., 2018).

The important issue to recognize here is that for all fresh produce it is not easy to investigate food illness associated with fruit due to normal handling and exchanging of hands as well as further processing before it reached the end consumer.

The most important step to extend shelf life of the different berry types includes cold chain maintenance, which normally involves either refrigeration or freezing. It was found that the survival of *Salmonella spp., E. coli O157:H7,* and *L. monocytogenes* on surfaces of unwashed and intact or cut strawberries were minimal for *L. monocytogenes* (1 to 3 logs) and no changes were observed in *E. coli* or *Salmonella* at ambient temperature (Sapers et al., 2009; Concha-Meyer et al., 2014 and Tavoschi et al., 2015). During refrigerated storage for seven days, all pathogen cocktail populations declined (1 to 3 logs) on intact strawberries, but no

population reduction was observed on cut surfaces (Sapers et al., 2009 and Concha-Meyer et al., 2014). Frozen storage for one month resulted in population reductions of approximately 2 logs or less, which gives as a clear indication that there was a high risk if contaminated berries for entering the food chain (Sapers et al., 2009 and Concha-Meyer et al., 2014). It is well established that bacterial pathogens are capable of survival through the normal shelf-life of all berry types regardless of storage temperature and that the sugar and acid content of these fruits also not served as a measure of protection against bacteria such as *E. coli, Salmonella* and *L. monocytogenes*, in addition not even washing of the berries before consuming had a real removal effect (Sapers, et al.,2009; Concha-Meyer et al., 2014 and Tavoschi et al., 2015).

The potential food poisoning risk associated with berries is ascribed to factors such as the popularity as a health food that led to increased production volumes and producers regarding it as a very high value commodity (Macori et al., 2018 and Concha-Meyer et al., 2014). Very little information is available about the accompanying microbiological risks of berries (Macori, et al., 2018 and Tavoschi et al., 2015). Mostly consumed raw or after minimal processing, berries and especially frozen berries was linked to food illness outbreaks and many factors were indicated in influencing food safety of berries, such as various approaches in the cultivating of berries (in soil, in pots, by hydroponic technologies, in open fields or in green houses), berry production also often formed part of exclusive or shared cultivation and farm size (Macori, et al., 2018 and Tavoschi et al., 2015). In Their study Macori et al (2018) highlighted the difficulty in detecting and preventing food poison outbreaks associated with berries and the difficulty in setting up surveillance systems to prevent microbial infections. The difficulty was linked to numerous risk factors associated with the contamination of berries, such as the type of cultivation used, the type of irrigation and water source and most importantly the fact that harvesting of berries was mostly done by hand, stressing the importance of hygiene awareness (Macori, et al., 2018 and Tavoschi et al., 2015). The evaluation of risk factors at primary production of berries was an important consideration in food safety. The identification of critical points at the primary production site were established with food safety audits (GAP), or physical microbiological evaluations of fruit, but due to the constraints in microbiological testing, unpredictability of microbiological

contamination, small sample size and cost, food safety principles combined with environmental scans remains the best options.

In their study Macori et al. (2018) developed an approach that is well suited to do environmental evaluation on potential microbiological risks on berries in South Africa as well. The approach was to develop a sampling plan based on background information gathered by questionnaires, and observations of all aspects and activities on the farms as to select berry samples from as many representative scenarios as possible (Macori et al., 2018; Tavoschi et al., 2015 and Van Schothorsta et al., 2008). A critical consideration in the Macori et al. (2018) study was hygiene training of the farm workers and practices during harvesting, as well as post-harvest handling operations, like washing, packaging, and storage. The information collected was critical in the interpretation of microbiological and the formulation of risk models for production units. The sampling plan for such an investigation would typically include a selection of producers, sampling methods and equipment, a method to transport samples and laboratory methods of analyses (Macori et al., 2018; Van Schothorst et al., 2008 and UFPA, 2010).

Macori et al. (2018) tested for the presence of *Salmonella, L. monocytogenes, E. coli* and Aerobic mesophilic count (AMC). The results of this study indicated that for blueberries the AMC was high (although there is no specified limit), the researchers linked this to the blueberry skin having a high resistance to handling (Macori et al., 2018). The pathogens *Salmonella* and *E. coli* (STEC) and the other targeted pathogens were not detected, leading the researchers to conclude that in terms of berries there was a low risk of foodborne pathogen contamination at the primary production stage and that fresh berries up to farm gate was relatively safe, despite irrigation water and soil samples often exceeding pathogen limits (Macori, et al., 2018). In terms of South African berry production at farm level this study could be valuable as guide for a similar risk assessment study, as studies of berry food safety risk were very limited as well as that the risk for contamination of berries in SA would probably give similar results.

2.6.2. Risk associated with Table Grapes.

Table grapes are one of South Africa's major export crops. Two major packing processes are popular in SA namely conventional pack house processes and vineyard packing, where grapes were packed directly into cartons in the vineyard one of the key reasons for this would be that there was less handling of the grape bunches, but as a result the risk of contamination from the environment increases substantially (NDA, 2012). Grapes were very sensitive to heat and moisture and to extend the shelf life, grapes need to be stored under cooling as quickly as possible (NDA, 2012). The drying out of stems and mould growth (*Botrytis cinerea*) were the two main reasons for post-harvest loss of shelf life and to mitigate this loss grapes were packed into perforated polyethylene plastic bags and Sulphur dioxide generating sheets were included in the carton releasing Sulphur Dioxide over the extended shelf life together with this a very strict cold chain protocol was in place for table grapes (NDA, 2012). Table grapes were non-climacteric fruit which means harvesting was done at optimal eating ripeness with a fully developed sugar content, relative low acid content and high moisture levels, making it an ideal environment for mould growth but also other micro-organisms (NDA, 2012).

Salmonella, E. coli and Listeria were linked with fresh table grapes, but a recent study by Carter, Feng, Chapman and Gabler (2018) indicated that both cold chain conditions during the export as well as the continual release of Sulphur Dioxide from the Sulphur Dioxide sheets significantly reduce the pathogen presence as well as their viability. It was also found that the combination of the polyethylene liner together with the Sulphur dioxide sheet and refrigerated temperatures provided an environment that very effectively reduced the presence of *L. monocytogenes, Salmonella enterica* and *E. coli* (O157:H7) (Carter et al., 2018).

2.6.3. Risk associated with Melons.

Melons were regularly associated with food borne disease, especially *L. monocytogenes* and *Salmonella* infections, although *E. coli* may also be associated (FSN, 2019). Melons grow on the ground, coming directly into contact with soil and irrigation water, increasing the potential for fruit surface contamination. In addition the melon surface is very rough increasing the potential for pathogens to attach to the rind, with a high possibility of forming

biofilms and even internalizing itself into the flesh of the melon (Danyluk et al., 2014; Scolforo et al.,2017; Spadafora et al., 2016 and Zhu et al., 2017). An important risk associated with melons and similarly fresh fruits such as avocados (FDA, 2018) was that although the contamination might have been restricted to the rind, which was not consumed, pathogens were transferred to the flesh during cutting or processing, especially *Listeria* outbreaks were linked with the spread of the pathogen from the surface to the flesh (Sapers, et al., 2009; Danyluk et al., 2014; Scolforo et al., 2017; Spadafora et al., 2016 and Zhu et al., 2017). Because of the high risk associated with pathogen presence on the melon surface as well as the melons capability to support the growth of pathogens due to mild acidity (pH 5.2 to 6.7) and a high relative water activity (0.97 to 0.99) the FDA classified it as a high risk or potentially hazardous food (Sapers, et al., 2009; Danyluk et al., 2014; Scolforo et al., 2017; Spadafora et al., 2016 and Zhu et al., 2017).

Melon contamination with pathogens mostly originates from the field during the growth phase through contact with contaminated soil, in additional to that contamination were also indicated to happen from contact of the melon rind with soil during harvesting and packing, or other sources such as water, equipment, and humans (Sapers, et al., 2009; Danyluk et al., 2014; Scolforo et al., 2017; Spadafora et al., 2016 and Zhu et al., 2017). Once into the consumer domain, melons were mostly precut and displayed, often without proper cold chain management increasing the risk of pathogen growth ((Sapers, et al., 2009; (Danyluk et al., 2014; Scolforo et al., 2017; Spadafora et al., 2016 and Zhu et al., 2017).

In conclusion when it comes to products such as melons and avocados where the surface provides opportunity for pathogens to survive, prevention of contamination remains the best option, which requires the producers and fruit handlers to be educated and made aware of the importance of GAP's as well as hygiene practices throughout the farm to the processing chain.

2.7. *E. coli, Salmonella* and *L. monocytogenes* as pathogens on fresh fruit

2.7.1. Focus on *E. coli* and Coliforms as a pathogen on fresh fruit.

Escherichia coli forms part of the *Enterobacteriaceae* family and is characterized as a catalase-positive, oxidase-negative, fermentative, short, Gram-negative, non-sporing rod (Yeni et al., 2016; Rajwar et al., 2016 and Adams and Moss, 2008). Genetically, *E. coli* was closely related to the genus *Shigella*, a pathogen also associated with fresh produce (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al., 2008). *E. coli* is associated with the human and other mammal digestive systems where it lives as a facultative anaerobe, together with a number of other micro-organisms as part of the intestinal microflora (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al., 2008). *E. coli* was considered harmless, but it became an opportunistic pathogen causing several infections such as Gram-negative sepsis, urinary tract infections, pneumonia, meningitis and neonates (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al., 2008). *E. coli* was found in animal faeces, it grows relatively easy on cultures, making it easy to isolate and with its generally less-pathogenic character, and survival characteristics in water *E. coli* became the preferred indicator organism for fecal contamination and therefore the presence of other enteric pathogens such as *Salmonella* (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al., 2008) . *E. coli* was widely used as indicator organism in food, the presence on or in food was considered significant in terms food safety and positive results on food would require further investigation (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al.,2008).

Over the last few years enterohaemorrhagic *E. coli* (EHEC) and specifically serotype O157:H7 was linked to various outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome in various countries all over the world and this serotype was linked to foods such as undercooked ground meat, raw milk and fresh produce (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al., 2008)**.** A further concern was that worldwide an exponential rise in isolations of O157:H7 was occurring and continues to do so, making *E. coli* of great importance when looking at fresh produce (Yeni et al., 2016; Rajwar et al., 2016 and Adams et al.,2008).

There were four major categories of diarrhoeagenic *E. coli* based on distinct, virulence properties namely:

Enterotoxigenic *E. coli* (ETEC) associated with symptoms ranging from mild diarrhea to symptoms representing cholera (Yeni et al., 2016 and Adams et al., 2008).

Enteroinvasive *E. coli* (EIEC). Infection result in symptoms typical for dysentery, symptoms were like that of *Shigella* (Yeni et al., 2016 and Adams et al., 2008).

Enteropathogenic *E. coli* (EPEC). Symptoms of EPEC infection include malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12–36h after ingestion of the organism. Infants were more prone to contracting this strain with more severe symptoms and persisting for a longer period (Yeni et al., 2016 and Adams et al., 2008).

Enterohaemorrhagic *E. coli* (EHEC). EHEC, also referred to as verotoxin-producing *E. coli* (VTEC), in many countries cause more outbreaks of diarrhoea than *Campylobacter* and *Salmonella, E. coli O157:H7* was the most common EHEC serotype reported (Yeni et al., 2016 and Adams et al., 2008). EHEC was important because foodborne transmission was more common than with other diarrhoeagenic *E. coli*, but also the severity which ranged from diarrhoea, to haemorrhagic colitis, to life threatening conditions namely haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura (Yeni et al., 2016 and Adams et al., 2008).

E. coli were associated with faecal contamination of water used in primary production and post-harvest processing as well as contaminated food handlers and was a good indicator of hygiene practices on a farm or in a pack house (Yeni et al., 2016 and Adams et al., 2008).

2.7.2. *Salmonella*

Data suggests that *Salmonella* survives for long periods of time on orchard floors, and fruit might become contaminated during harvesting operations (Sapers, et al.,2009; Adams et al., 2008; Rajwar et al., and Yeni et al., 2016). *Salmonella* were characterized as Gram-negative bacteria of the *Enterobacteriaceae* family normally present in the gut of certain animals particularly birds, especially domestic birds such as chickens as well as in reptiles (Sapers, et al.,2009 and Adams et al., 2008). Humans get infected with Salmonella through eating of contaminated food products, and the size of the infecting dose was critical in terms of the severity of the infection (Sapers, et al.,2009 and Adams et al., 2008). *Salmonella* infection was typically associated with meat, poultry, milk and eggs but cases of contaminated fruit was

reported on more regularly now (Sapers, et al.,2009; Adams et al., 2008, FDA, 2019 and FSN, 2019). Non-typhoidal *Salmonella* usually just causes gastrointestinal disease such as diarrhea and abdominal cramps until the body's natural immune system overcomes the infection, of importance was that people with compromised immune systems might not overcome even a mild infection (Sapers, et al.,2009; Adams et al., 2008; Rajwar et al., and Yeni et al., 2016). Sometimes *Salmonella* organisms invaded other parts of the body and penetrate other organs that can lead to other infections such as meningitis or liver abscesses (Sapers, et al.,2009; and Adams et al., 2008). Disease caused by non-typhoidal *salmonella* was called "salmonellosis", while typhoidal *Salmonella* cause a disease called typhoid fever, which was indicated as life-threatening, especially to people with weak and compromised immune systems (NICD, 2016).

Salmonella was very closely associated with the agricultural environment and in South Africa *Salmonella* was commonly found associated with livestock and poultry, meat at abattoirs, raw materials at feed mills, animal feed, and environmental sources especially water. *Salmonella* outbreaks occur frequently in South Africa especially during summer and it was reported to be more common amongst the poorer socio-economic households (NICD, 2016).

Symptoms of salmonellosis include diarrhea, abdominal cramps and fever with occasional symptoms of chills, headache, nausea and vomiting (Sapers, et al.,2009; Adams et al., 2008; Rajwar et al., and Yeni et al., 2016). The onset of illness occurs between 6-72 hours, depending on the infection dose, after eating contaminated food (Sapers, et al.,2009; Adams et al., 2008; Rajwar et al., and Yeni et al., 2016). Healthy persons contracting a mild infection could recover completely without treatment relatively quickly (Sapers, et al.,2009; Adams et al., 2008; Rajwar et al., and Yeni et al., 2016). Salmonella causing meningitis, pneumonia and abscesses were regularly reported and these conditions were more life threatening (NICD, 2016).

2.7.3. Listeria

Listeria monocytogenes was characterised as a Gram-positive, facultatively anaerobic, catalase positive, oxidase-negative, non-encapsulated, non-spore former with coccoid to rod shaped cells and possess peritrichous flagella (Adams et al., 2008; Zhu et al 2017; Lawley, Curtis and Davis, 2012). The *Listeria monocytogenes* bacterium was found to be widely

distributed in nature and was commonly found in soil and water and regularly reported on as a pathogen associated with fresh produce (Adams et al., 2008; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). Infection with *Listeria* usually results in gastro-enteritis with symptoms ranging from mild to severe depending on the infection dose (Adams et al., 2008; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). People with weak immune systems were identified as at highest risk when infected with *Listeria,* infection with *listeria* causes Listeriosis which can lead to secondary infections such as meningitis or septicaemia (Adams et al., 2008; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). Pregnant women were identified as the highest-risk group and Listeriosis may result in miscarriage or meningitis of their infant (NICD, 2017, Adams et al., 2008; Smith, Hearn, Taylor, Wheelhouse, Kaczmareka, Moorhouse and Singleton, 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016).

L. monocytogenes was linked to growing vigorously on or in a variety of surfaces and mediums including water, soil, stainless steel surfaces and foods such as meat but were isolated from fresh fruit and vegetables internally as well as on the surface (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). The optimal growth temperature was established at 30-37°C but *listeria was also capable of* growing at temperatures as low as 4°C (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). Growth has been observed at temperatures as low as -1.5°C and as high as 45°C (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). To effectively inactivate *L. monocytogenes,* researchers recommends heat treatments of 70°C or more for at least 2 minutes (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). Researchers also established that *listeria* was able to survive in very low water activity conditions and live in a pH environment of between 4.3-9.4 and was also able to survive in high salt concentrations (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). Because of the close association of *L. monocytogenes* with soil and water, as well as a well-documented close association with vegetation and animals, *listeria* was identified as a common presence in agricultural production sites and processing environments (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). *L. monocytogenes* were identified as a transitory bacterium in the digestive tract of humans and animals, with up to 10% of the population being carriers or hosts without health implications (Adams et al., 2008;

Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). *L. monocytogenes* was indicated as being able to encapsulate itself with a slimy layer protecting itself against harsh environmental conditions (Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017; Lawley et al., 2012 and Yeni et al., 2016). The major concern with *L. monocytogenes* in terms of food safety and in relation to fresh fruit was its high tolerance to environmental conditions such as high salt, high acidity, low oxygen and low temperatures, and therefore, the ability to survive for long periods of time in processing and household environments (Lawley et al. 2012; Robinson et al., 2000; CAC/GL 61-2007; Adams et al., 2008; Smith, et al., 2019; Zhu et al 2017 and Yeni et al., 2016).

Chapter 3: Materials and methods

The data for this study was obtained via the PPECB standards co-ordination desk which was the link between PPECB and DALRRD. Indonesia microbiological, MRL and heavy metal samples were drawn by PPECB inspectors as indicated in the sampling methodology (3.1) and couriered to the designated accredited laboratory. After analysis of the samples, laboratory results were sent to DARDLR and PPECB, DARDLR would then issue a food safety certificate. PPECB verify compliance and then issue and export certificate or rejection note per consignment (normally 20 pallets of a specific product, that would be loaded into a refrigerated shipping container). These microbiological, MRL and heavy metal results were tabulated and stored by DARDLR and PPECB since the inception of the Indonesia SOP in 2014. The results in this study represents microbiological data from 2014 up to the end of 2019 deciduous season.

3.1 Sampling methodology and sampling size for fruit.

Background

The samples for microbiological testing for fruit destined for Indonesia were drawn by PPECB inspectors on instruction from specific exporters. These instructions include the product, Production unit code (PUC - producer), cultivar and orchard number that would be indicated on the Indonesia export certificate as proof of the correct batch of fruit tested and that it could be linked to the food safety certificate issued by DARDLR, traceability remains critical throughout the process.

After instruction was received the designated PPECB inspector would then identify the consignment of fruit packed in cartons on pallets as part of the inspection sample (in other words the fruit was sampled after harvesting and all pack house operations). This was important to ensure that the sampling was done after all possible points of microbial contamination.

The inspector would then sterilize his or her hands by washing with soap and water and using a sanitizer. This process was not monitored and was dependent on inspector training and adherence to the sampling SOP. The sampling process might therefore have been a potential contamination point as well. The inspector would then take a new unused plastic sample bag (sample bags were not sterile, just new and never been used before) and put at least 1kg of fruit into the sample bag, the bag would then be sealed by folding the top over a few times and using a stapler to close it down. Stickers containing all the relevant information would then be placed on the bag, samples were grouped at the local PPECB office, placed in cooler boxes and couriered to the relevant accredited laboratory for analyses. The process for drawing samples was documented in PPECB internal work instructions based on ISO 7002:1986 and the Indonesian SOP (DAFF, 2016).

3.1.1. ISO 7002:1986, Agricultural food products -- Layout for a standard method of sampling from a lot

Samples for microbiological analysis was drawn and prepared according to the Standard operating procedure on sampling and analysis of agricultural products of plant origin to determine agro-chemecical residue levels and risk management as part of export inspection and certification in terms of the APS act 119 of 1990, in accordance with ISO 7002:1986 principles (DAFF, 2019).

In the SOP "**bulk sample/aggregate sample**" (also referred to as the "inspection sample" in the export standards and requirements) was defined as the combined and well-mixed aggregate of the primary samples taken from a consignment, in terms of fruit a 2% sample of all cartons in consignment or on a pallet in the case of pallet inspection, Indonesia samples were drawn on a consignment principle (DAFF, 2019).

The primary (2%) sample provided enough material to enable the laboratory samples to be drawn (DAFF, 2019).

" A **consignment**" was defined as a quantity of a specific agricultural product of plant origin which -

(a) belongs to the same owner, was delivered at the same time under cover of the same delivery note, consignment note or receipt note, or was delivered by the same vehicle;

(b) or subdivided into different cultivars, classes, sub-classes, grades, types, counts, count groups, type groups, size groups, colour groups, diameter groups, production groups, diameter codes, size codes, production lots, pallet loads, trademarks, packaging sizes or types of packaging in every quantity of each of the different cultivars, classes, sub-classes, grades, types, counts, count groups, type groups, size groups, colour groups, diameter groups, production groups, diameter codes, size codes, production lots, pallet loads, trademarks, packaging sizes or types of packaging" (DAFF, 2019).

To determine the design and implementation of a sampling programme, according to ISO 7002:1986, the following consideration was factored in: shipment size (in the case of fruit inspected on consignment base, normally 20 pallets, the number of cartons would have varied depending on fruit type). Inspectors would have drawn a 2% sample of cartons from the total amount of the cartons in the inspection sample. From the 2% cartons at least 1kg of fruit was taken from more than 1 carton, placed in new plastic bag and sealed for dispatch in a cooler box. Fruit variability (fruit of same size and cultivar), laboratory accuracy, cost of the assay and value of the fruit, blueberries have a much higher value than citrus must also be considered. Sampling protocols must meet scientifically recognized principles and procedures. No sampling equipment is needed in the case of fruit. The sample process therefore was extremely simple and independent, results and data were trustworthy because the producer or pack house could not have influenced the result in any way. In this respect the sampling process for the purposes of this study was compliant. Statistically the sample represents a very small sample of fruit from an orchard. Not all fruit could be sampled, making it necessary to follow a sampling plan that could give a high confidence in the results.

Sampling process:

Precautions that were stipulated but impractical to monitor

(a) During sampling of agricultural plant products for analytical purposes, every precaution was taken to prevent contamination and deterioration of the samples or subjecting the samples to such changes that the residue/microbiological content thereof was affected – all samples were dispatched in cooler boxes (DAFF, 2005).

(b) Each laboratory sample sent to the laboratory should have represented the consignment destined for Indonesia, inspectors should have therefore taken every precaution to make sure the samples were drawn from the correct sample boxes (DAFF, 2005).

Collection of primary samples ("sample of the consignment")

(a) Each primary sample was taken from a randomly chosen position in the consignment, as far as practically possible (DAFF, 2005).

(b) The primary samples must consist out of enough material to provide the laboratory sample(s) required (DAFF, 2005).

(c) The minimum number of primary samples that were taken from the consignment were as follows in cases where --

(d) plant products, either packaged or in bulk, were assumed to be well mixed or homogeneous:

The total number of cartons or other types of containers in the consignment was known:

Number of containers in the consignment, minimum number of primary samples that was taken from the consignment: 1 – 25 (1 carton), 26 – 100 (5 cartons), > 100 (10 cartons) (DAFF, 2005)

Preparation of the bulk sample ("inspection sample")

The primary samples were combined and/or mixed well, if practically possible, to form the bulk sample (DAFF, 2005).

Preparation of the laboratory samples

(a) Laboratory samples were taken randomly from the bulk sample (DAFF, 2005).

(b) Where the bulk sample was larger than was required for a laboratory sample, it was divided to provide a representative portion. Quartering, or other appropriate size reduction processes were used but units of fresh plant products were not cut or broken (DAFF, 2005).

(c) The minimum size required for laboratory samples was as follows:

Fresh products of plant origin (All fresh fruit and vegetables) all fruit 1kg, accept grapes (2kg)

A smaller laboratory sample was taken from a product of exceptionally high value, for example blueberries, provided that the reason(s) for doing so was noted in the sampling record (DAFF, 2005).

3.1.2. Handling and dispatch of samples as per DAFF MRL SOP

General:

(a) The person responsible for taking the samples were trained to always wash their hands prior to sampling (DAFF, 2005).

(b) Samples were handled as little as possible and inspectors were trained to put the samples into the plastic bags or containers in which it will be dispatched as soon as possible (DAFF, 2005).

(c) All samples were placed in clean plastic bags (provided by PPECB) which were new, large and strong enough to ensure that the samples were delivered intact to the laboratory. A hard copy of the inventory was included in the container. Care was taken not to overfill the plastic bags (DAFF, 2005).

(d) Plastic bags or containers in which samples were placed were sealed to prevent contamination from the outside. Each plastic bag was folded down repeatedly to remove the air inside as far as possible and was stapled on the folds (DAFF, 2005).

(e) To prevent damaging of the fruit or plastic bags, not too many samples were dispatched in the same outer container (DAFF, 2005).

(f) Samples were as far as possible not exposed to high temperatures and were, where possible, stored under refrigeration or at least in a cool place before being dispatched to the laboratory (DAFF, 2005).

(g) Samples reached the laboratory in a good condition within 2 working days after sampling, as per the PPECB SOP (DAFF, 2005).

(h) Samples were stored out of direct sunlight at the laboratories (DAFF, 2005).

3.2. Analyses done by accredited laboratories

3.2.1. ISO 6887-1:2017

This microbiology method was designed for the food chain and involves the preparation of test samples, initial suspension and further decimal dilutions from the initial suspension, for microbiological examination and include the general rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1, 2017).

After receiving the samples, the first step was the preparation of an initial suspension. In the case of fruit this process was the pulping of the whole fruit and then taking 10g of this homogenous pulp and diluting it in 90g of distilled water (nine -fold quantity of dilatant) to obtain a 10-fold dilution. Further dilution with a peptone salt solution was then done to obtain the suspension that was inoculated on the appropriate medium (ISO 6887-1:2017). This initial suspension will be used for further analysis for *Salmonella* and *E. coli* according to the ISO methods.

3.2.2. International standards for testing microbiological contamination of fruit samples

The Indonesia samples were only tested for *Salmonella* and *E. coli* as described below

3.2.2.1 *Salmonella*:

"The Rapid *Salmonella* (ISO 6579-1 (April 2017))" method to detect the presence of *Salmonella* also referred to as a horizontal method for the detection of *Salmonella,* was used by the accredited laboratories (Mooijman, 2018 and ISO 6579-1, 2017). This method was the preferred method for the detection of *Salmonella* in the food industry and was applicable to the following, food intended for human consumption and animal feed, and environmental samples from food production sites and food handling facilities (Mooijman, 2018, ISO 6579-1, 2017). This method was also used to determine the presence of *Salmonella* in samples from the primary production stage such as animal faeces, dust, and surface swabs (Mooijman, 2018 and ISO 6579-1, 2017).

This method was designed to detect most of the *Salmonella* serovars and for the detection of some specific serovars, additional culture steps were needed (Mooijman, 2018 and ISO 6579-1, 2017).

The detection of *Salmonella* required 4 successive stages

The first stage involved the pre-enrichment in a non-selective liquid medium. A buffered peptone water solution at ambient temperature was inoculated with the test portion obtained according to the method in ISO 6887-1:2017 to 10-fold dilution and incubated at a temperature between 34°C and 38°C for 18 hours (ISO 6579-1, 2017).

The next step involves the selective enrichment of the media obtained in the first step, Rappaport-Vassiliadis medium with soya broth and Muller-Kauffmann tetrathionate-novobiocin broth were inoculated with the culture obtained in the pre-enrichment step for 24h at 41,5°C in the soya broth and for 24h at 37°C in the Muller-Kauffmann broth (ISO 6579-1, 2017)..

The next step was the plating out of the culture obtained in the second phase on selective solid media namely Xylose Lysine Deoxycholate (XLD) agar and any selected medium complementary to XLD agar for 24H at 37°C.

The last step was the confirmation step where colonies of presumptive *Salmonella* were sub cultured and their identity confirmed by biochemical and serological tests. Confirmation of at

least 1 suspect colony obtained from the last step must be positive, if negative 4 additionally suspect colonies had to be confirmed (ISO 6579-1, 2017).

3.2.2.2. *Escherichia coli:*

For *E. coli* the ISO 16649-2 method was used. ISO 16649-2 relates to the microbiology of food and animal feeding stuffs to determine the presence of *E. coli*. This method makes use of the horizontal enumeration of ß-glucuronidase-positive-*Escherichia coli*- and the Colony-count technique at 44°C using 5-brom-4chloro-3-indolyl ß-D-glucuronide (ISO 16649-2 and AOAC, 2016).

Duplicate sterile petri dishes were inoculated with 1 ml each of the test sample from the initial suspension (10-fold dilution) obtained according to the method prescribed in ISO 6887-1:2017. 15 ml of tryptone-bile-glucuronic medium (TBX) cooled to 45°C (+ or - 1°C) were added to each petri dish, the TBX was allowed to solidify, inverted and incubated at 37°C (+ or - 1°C) for 4 hours, followed by at 44°C (+ or – 0.5°C) for 18-24 hours (ISO 16649-2 and AOAC, 2016). An optional resuscitation stage at 37°C for 4 hours could be used to allow for recovery of sublethal injured cells (ISO 16649-2 and AOAC, 2016). The petri dishes were then examined to detect the presence of colonies with the characteristic properties of ß-glucuronidase-positive *Escherichia coli,* typically blue colonies were counted and used to determine CFU/g (ISO 16649-2 and AOAC, 2016).

3.2.2.3 *Listeria monocytogenes*:

Rapid test for *Listeria monocytogenes* (ISO 11290-2 absence test). This method was used to determine presence of *Listeria monocytogenes* in the environmental microbiological results from a blueberry farm.

This method requires 5 successive steps, the first step was the preparation of the initial suspension as described in ISO 6887-1:2017, a 10-fold dilution in buffered peptone water or alternatively Half-Fraser broth supplemented by selective agents could also be used, this diluted suspension was used for both the detection and enumeration steps for *Listeria monocytogenes*. If Half-Fraser broth was used plates must be inoculated within 45 minutes (ISO 11290-2, 2017).

The next step was to inoculate 0.1ml of the suspension and 0.1ml of further dilutions, if required, on "Agar *Listeria"* (ISO 11290-2, 2017). In situations where a low numbers of Listeria monocytogenes was suspected, the limits of detection could be raised by a factor of 10, by increasing the inoculate to 1ml. The ISO 12290-2 method advises that it was best practice to prepare duplicate plates. The inoculum was spread as quickly as possible over the surface of the agar *Listeria* without touching the sides of the petri dish with a spreader, a fresh spreader was used for each dilution, the plates were then left closed and upright for 15 minutes at ambient temperature for the inoculum to be absorbed into the agar *listeria* (ISO 11290-2, 2017).

The next step was to incubate the Agar *listeria* plates inverted at 37 °C for 24 hours (+ or – 2 hours), and additional incubation at the same temperature and time period was allowed if required (ISO 11290-2, 2017)

The next step was the enumeration of characteristic colonies, After incubation for 24 hours, if *Listeria monocytogenes* was present, before excessive development of colonies with large and overlapping halos formed, which would make reading difficult, or if poor colony development, a further 24 hour incubation was allowed, the dishes was examined for the presence of presumptive colonies of *Listeria* monocytogenes (ISO 11290-2, 2017).

Listeria monocytogenes was identified by the blue-green colonies surrounded by an opaque halo, *Listeria ivanovii* looks similar, L. monocytogenes under acid stress would have a very weak halo, and some rare strains of L monocytogenes will require 4 days of incubation due to slow phosphatidyl inositol phospholipase C activity (ISO 11290-2, 2017).

The next step was to count all colonies presumed to be Listeria monocytogenes on each petri dish containing less than 150 colonies (90mm dish) or less than 360 colonies (ISO 11290-2, 2017).

The next step was the confirmation step, from each petri dish used for the initial suspension, containing presumptive colonies take 5 colonies, representing each colony type (halo size and blue-green coloration) and streak the selected colonies onto the surface of pre-dried plates

of non-selective agar (blood agar, nutrient agar or tryptone soya yeast agar (TSYEA)) and incubate at 37°C for 18-24 hours or until the growth was satisfactory (ISO 11290-2, 2017).

The last step was to select colonies from the non-selective agar and by means of appropriate morphological, physiological or biochemical characteristics confirm *L. monocytogenes.* From the number of confirmed colonies, the calculation of the number of *L. monocytogenes and/*or *Listeria spp., were* expressed as per gram or milliliter (ISO 11290-2,2017).

3.2.2.4. Aerobic Plate Count (APC), Total plate count (TPC) or Total Bacterial plate count (TBC)

Total bacterial counts are typically part of environmental studies as part of the required set of microbiological results as an indicator for hygiene effectiveness and formed part of the results of the blueberry farm.

APC/TPC/TBC was used as an indicator of the total number of bacteria in a food product. APC only measures microorganisms capable of growing at 30-37°C in the presence of oxygen and were typically incubated at 35±1°C for 48±3 hours, although other temperatures (e.g. 25°C) was also used (UFPA, 2010 and AOAC, 2019). Mostly these Aerobic Plate Counts resulted in counts within very high numbers depending on the commodity and other production circumstances (UFPA, 2010 and AOAC, 2019). These organisms cannot normally grow at the low temperatures used for storing fresh and fresh-cut produce, and few can grow in an oxygen-depleted atmosphere (UFPA, 2010 and AOAC, 2019). It is important to remember that many of the organisms that could grow at low temperatures are not able to grow at the higher temperature used for the APC test and that microorganisms detected by APC were usually not pathogens, APC results therefore do not correlate well with the potential for pathogen contamination and is not useful predictors of product safety (UFPA, 2010 and AOAC, 2019). Total plate counts were used to measure trend analysis of finished product microbial ecology, since it gives a good indication as an environmental indicator of sanitation processes and process control and were a good indication that microbiological quality of the product may be unacceptable (UFPA, 2010 and AOAC, 2019). TPC was not used as an indicator of safety or the presence or absence of pathogens, as a routine indicator of initial quality or when baseline studies demonstrate that product or environmental conditions normally have a wide range of microbial populations (UFPA, 2010 and AOAC, 2019).

Based on the results of the discussed methods the following limits were applied.

Table 3.1: Microbiological limits for Salmonella, E. coli and Listeria

(Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs)

Microorganism	**Food**	**Limit at Production unit**	**Limit at market**	**Test method**
L. monocytogenes	Ready to eat food (including fresh fruit and vegetables) able to support *L. monocytogenes*	Absence in 25g	100 cfu/g	ISO 11290-2 (absence testing)
Salmonella	Ready to eat fresh fruit and vegetables	Absence in 25g	Absence in 25g	ISO 6579
E. coli	Ready to eat fresh fruit and vegetables	100cfu/g (2 samples can have up to 1000cfu/g provide that more than 5 samples are tested and the other samples are below 100cfu/g)	100cfu/g (2 samples can have up to 1000cfu/g provide that more than 5 samples are tested and the other samples are below 100cfu/g)	ISO 16649-1/2

Chapter 4: Results

4.1. Indonesia microbiological results 2014-2019

The results summarized in Table 4.1.1 represent all the fruit samples collected by PPECB for microbiological analyses for Indonesia since 2014 up to the 2019 deciduous season. Indonesia required that only *Salmonella and E. coli* were tested for, apart from the MRL and heavy metal analyses. Table 4.1.1 indicate the fruit type, year of analyses and the number of samples that was send to the laboratory and every sample represents one consignment of fruit. The number of representing PUC's, in some cases more than one fruit sample was drawn at one specific PUC during the production season, that would explain why there was in some instances more tests than representing PUC's. The next two columns indicate whether *Salmonella* or *E. coli* were found during analysis and how many samples tested positive. Not Detected (ND) indicate that no colonies of the bacteria were detected, this was further explained in table 4.1.2. The last column gives an indication of production areas which were covered during sampling. The Eastern Cape (EC) represents the major production area for citrus (Patensie, Sundays river valley and Fort Beaufort), and pome and stone fruit (Langkloof). Limpopo and Mpumalanga represent major production areas for Citrus. The Western Cape (WC) represents the major production areas for deciduous fruit grapes, pome and stone fruit, with Citrusdal also a major citrus production area. Other areas such as Gauteng also produce some of the fruit in small quantities that was exported from occasionally. Results indicated as $\leq$1 CFU/g were not considered. Table 4.1.1 represents a summary of the full set of results.

Table 4.1.1. Summary of Indonesia results

Product	**Year**	**No of samples send for analyses**	**No of PUC's represented**	***E. coli***	***Salmonella***	**Production areas represented**
Citrus	2014	15	15	ND	ND	EC/Limpopo/Mpumalanga
	2015	17	17	ND	ND	EC/Limpopo/Mpumalanga
	2016	1	1	1 with a result ≤1 CFU/g	ND	
	2017	16	16	ND	ND	EC/Limpopo/Mpumalanga
	2018	117	95	ND	ND	EC/Limpopo/Mpumalanga
Apples	2014	30	26	ND	ND	EC/Elgin/Ceres/WC other
Apples	2015	56	44	42 result with ≤ 1 CFU/g Rest ND	ND	EC/Elgin/Ceres/WC other

Product	**Year**	**No of samples send for analyses**	**No of PUC's represented**	***E. coli***	***Salmonella***	**Production areas represented**
	2016	155	108	ND	ND	EC/Elgin/Ceres/WC other
	2017	52	50	ND	ND	EC/Elgin/Ceres/WC other
	2018	124	62	ND	ND	EC/Elgin/Ceres/WC other
Pears	2014	194	134	2 with a result ≤1 CFU/g Rest ND	ND	EC/Elgin/Ceres/WC other
	2015	366	194	40 with a result ≤1 CFU/g 3 samples tested + Rest ND	ND	EC/Elgin/Ceres/WC other

Product	**Year**	**No of samples send for analyses**	**No of PUC's represented**	***E. coli***	***Salmonella***	**Production areas represented**
	2016	447	221	100 with a result ≤1 CFU/g Rest ND	ND	EC/Elgin/Ceres/WC other
	2017	323	243	ND	ND	EC/Elgin/Ceres/WC other
	2018	392	247	ND	ND	EC/Elgin/Ceres/WC other
Grapes	2014	61	46	ND	ND	Gauteng/Hex/Paarl/Piket/NC
	2015	113	59	ND	ND	Gauteng/Hex/Paarl/Piket/NC
	2016	39	29	37 with a result ≤1 CFU/g	ND	Gauteng/Hex/Paarl/Piket/NC

Product	**Year**	**No of samples send for analyses**	**No of PUC's represented**	***E. coli***	***Salmonella***	**Production areas represented**
	2017	69	57	ND	ND	Gauteng/Hex/Paarl/Piket/NC
	2018	64	48	ND	ND	Gauteng/Hex/Paarl/Piket/NC
	2019	18	16	ND	ND	Gauteng/Hex/Paarl/Piket/NC
Pome	2019	19	19	ND	ND	EC/Elgin/Ceres/WC other
Stone		2	2	ND	ND	Western Cape
Raisins		2	2	ND	ND	Northern Cape
Total		2688 samples tested	1747 PUC's represented	3 + Results	ND	

Table 4.1.2. The 3 positive results of *E. coli* detected on Pears analysed

Date	**PUC (Production area)**	***E. coli* result**
28/04/2015	C1150 (Ceres)	≤6 cfu/g
10/03/2015	E0059 (Grabouw)	≤19 cfu/g
15/09/2015	H0859 (Villiersdorp)	≤2 cfu/g

Table 4.1.2 represents the 3 significant positive results for E. coli, notably important about these positive results was that it only occurred during 2015 and all in the WC production areas. The unique PUC's represents 3 different farms, the PUC represents the farms registration code with DARDLR. Despite the positive results the presence of *E. coli* was still within acceptable levels for Indonesia.

4.2. Environmental case study of Blueberries as an example of pro-active compliance to Food safety certification.

Blueberry Mountain a Blueberry farm in the George area provided environmental microbiological results for the 2018 season. Samples were analyses by and accredited laboratory. These results serve to illustrate requirements for exporting farms to prepare for GlobalG.A.P. and BRC food safety audits. These results were only available to the food safety auditor and not to any other authority to determine compliance. Food safety auditors signs agreements of confidentiality, binding them to non-disclose of these results even in a case of non-compliance and only allows for the testing of corrective actions taken by the farm or pack house audited.

Table 4.2.1 Workers hands (Field Pickers, after following the hand cleaning procedure):

Test	Test Date	Result	Unit
Worker 1			
Escherichia coli (method SWJM 62/ISO 16649-2)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49/ISO 21528-2)	01/08/2018	No growth	cfu/area
Salmonella (SWJM 67/ISO Method 6579)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53/ISO 6888-1)	01/08/2018	No growth	cfu/area
Worker 2			
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area
Worker 3			
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area

Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area

Represents randomly selected field workers, responsible for picking blueberries in the orchards. Hand swaps were taken after they followed the hand cleaning procedure which includes washing hands with soap and water and then sanitizing with a hand sanitizer.

Table 4.2.2. Field picking truck in operation – unwashed interior wall surface

Test	**Test Date**	**Result**	**Unit**
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Listeria monocytogenes (Method SWJM 23/ISO 11290-01)	01/08/2018	Absent	cfu/area
Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area
TMA (method SWJM 35/ISO4833)	01/08/2018	30*	cfu/area

*A TMA count of 30 cfu/area was still within the limits

Represents the analysis of a sample taken of the interior walls of the truck responsible for transporting the blueberries from the orchard to the packing

facility. This truck makes several trips during the day and only get sanitized the morning before picking activities commence.

Table 4.2.3. Field picking station – unwashed

Test	Test Date	Result	Unit
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Listeria monocytogenes (Method SWJM 23)	01/08/2018	Absent	cfu/area
Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area
TMA (method SWJM 35)	01/08/2018	110*	cfu/area

** Results indicate that action was required in terms of better cleaning procedures, these results would have prompted an investigation by the producers, the producer would have demonstrated to the auditor that he noticed the exceedance and the auditor would have asked for proof of action and possibly follow up results.*

Represent a picking station a trolley designed to carry empty and filled buckets of blueberries moved around by the pickers, when all the picking buckets were filled the station would be moved to the transport truck, filled buckets will be delivered end empty buckets will be loaded on the station, the sample was taken from the surface, these stations were also only sanitized the morning before activities starts.

Table 4.2.4. Field picking tap water

Test	Test Date	Result	Unit
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Listeria monocytogenes (Method SWJM 23)	01/08/2018	Absent	cfu/area
Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area
TMA (method SWJM 35)	01/08/2018	10	cfu/area

Represents a sample of the tap water available to field pickers for sanitation purposes, hand washing and for general water requirements.

Table 4.2.5. Field picking bucket surface and handle (washed in pack house before use)

Test	Test Date	Result	Unit
Escherichia coli (method SWJM 62)	01/08/2018	No growth	cfu/area
Enterobacteriaceae (method SWJM 49)	01/08/2018	No growth	cfu/area
Listeria monocytogenes (Method SWJM 23)	01/08/2018	Absent	cfu/area

Salmonella (ISO Method 6579 – SWJM 67)	01/08/2018	Absent	cfu/area
Staphylococcus aureus (Method SWJM 53)	01/08/2018	No growth	cfu/area
TMA (method SWJM 35)	01/08/2018	No growth	cfu/area

Field picking buckets were used to place the picked blueberries in for transport from the orchard to the packing facility, the surface of the bucket was a critical point of concern as this surface comes directly into contact with the fruit. Sanitizing of picking buckets takes place after every delivery at the packing facility before it was sent back to the orchard.

Table 4.2.6. Fruit test Yeasts and moulds (on blueberries)

Sample 1	**Method**	**Date**	**Result**	**Unit**
Yeast	SWJM 50	15/08/2018	No growth	cfu/g
Mould	SWJM 50	15/08/2018	270	cfu/g
Sample 2				
Yeast	SWJM 50	15/08/2018	3700	cfu/g
Mould	SWJM 50	15/08/2018	No growth	cfu/g

Represents a sample of the blueberries itself that was tested for yeasts and moulds, one would expect positive results as yeast and mould were associated with fruit surfaces and would give an indication of yeasts and moulds in the environment as well as potential shelf life problems.

Table 4.2.7. Fruit tested at arrival on overseas market (Intertek – UKAS 4065) 29/08/2018

Organism	**Method**	**Result**	**unit**
Beta-glucuronidase positive *E. coli*	M012	≤10	cfu/g
Coagulase positive *Staphylococci*	M014	≤20	cfu/g
Detection of *Listeria* spp. by ELISA	M035	Not detected	In 25g
Detection of *Salmonella* spp. by ELISA	M041	Not detected	In 25g

Represents the final analysis that was done by the importer of the blueberries as it arrived at the overseas market, demonstrating the precautions taken on overseas markets to comply to food safety compliance.

Chapter 5: General discussion and conclusions

5.1. Discussion Indonesia results.

South Africa currently has 5010 farms registered with DAFF, exporting fresh fruit and vegetables to international markets (Foodtradesa, 2019). Fresh fruit and vegetables are packed in 850 registered pack houses spread through all the production areas of SA (Foodtradesa, 2019). The major export products include citrus, table grapes, pome fruit, stone fruit and avocados followed by numerous minor products such as blueberries and various other berry types, pomegranates, sub-tropical fruit (mangoes and litchis) and various vegetable types (for example butternuts, onions, potatoes) (Foodtradesa, 2019). The major export regions of SA include the Western Cape, Northern Cape, Eastern Cape, Limpopo and Mpumalanga, KwaZulu-Natal and various small productions areas such as Gauteng (Foodtradesa, 2019). Fruit mostly are exported via containerized shipping or airfreight (Foodtradesa, 2019).

For farms or production units and pack houses to form part of the export chain they need to comply to food safety requirements and this must be proofed with a valid commercial GAP system such as GlobalG.A.P and HACCP or at least the basic food safety audits provided by PPECB (Notice 707, 2015). The basic food safety audits come with limited market access and was not as strict or expensive as the commercial options. In SA pack houses were grouped in two main groups, on farm pack houses, where the producers invested in their own packing facility or co-operative pack houses, where a group of producers pack their fruit together. On-farm pack houses were included in the GlobalG.A.P audit, but off-farm pack houses had to have a HACCP, BRC or at least the SA-off farm equivalent in place. Many on-farm packing facilities also invested in HACCP or BRC, to get a marketing advantage. Food safety in the SA environment was dependent on the effectiveness of the food safety auditors, food safety systems such as Global G.A.P depends on good auditing techniques. Food safety systems depends on how truthful the producer was in presenting his system and results, including critical components such as microbiological analysis of water and the environment, to the food safety auditor.

The only independent samples drawn for microbiological analysis on fresh fruit on a consignment basis were the samples drawn by PPECB for microbiological testing on

consignments of fruit exported to Indonesia, a protocol that started in 2014. Indonesia exports were limited to citrus, pome fruit (apples and pears) and table grapes with only occasional exports of other fruit types.

Citrus fruit was not included in the protocol for microbiological analysis but only for MRL and heavy metals, since citrus was deemed low risk for microbiological contamination due to the thick flavedo and albedo and the fact that the skin was not consumed, and internalization of microbes was unlikely. Microbiological results on citrus was available due to inspectors following through on the complete sampling SOP (166 test representing 144 PUC's, 2014-2018), with no *Salmonella* or significant *E. coli* being detected. Although this was a small number of tests compared to the total volume of fruit being exported it does cover the main production areas and support the idea that citrus fruit was low risk in terms of microbiological contamination. Citrus fruit goes through a washing (chlorine bath) as well as being waxed before being packed in export cartons, the only microbiological contamination that was most likely to occur is through pack house hygiene relating to the cleanliness of packing surfaces and packer hygiene, effectiveness of hand washing and water quality.

Between 2014 and 2018, 417 apple samples representing 278 PUC's were drawn for Indonesia. The samples represented all production areas. The microbiologically results indicated that none of the samples contained *Salmonella* or significant (≤ 1) *E. coli* and although this represents a small number of samples against the total export volume and amount of PUC's producing apples, it gives an indication that apples were also microbiologically safe.

Pears were by volume the most important fresh fruit exported from SA to Indonesia and between 2014 and March 2019 a total number of 1722 of samples were tested for *Salmonella* and *E. coli* representing 1039 production units, which was a small number of samples compared to the total amount of Pears exported to al markets. Compared to the other products pears were the only fruit type where *E. coli* was present on 3 samples, during 2015, these findings occurred in various Western Cape production areas (Ceres, Grabouw and Villiersdorp). The amount cfu/g detected for *E. coli* still did not exceeded the maximum number allowed (Table 3.1). The question why *E. coli,* although still within limits was only

found on pears, could possibly be answered by the pear surface which was not as smooth compared to apples, and this rougher surface provide better adhesion opportunity for the *E. coli* bacteria. Linked to the first reason pear shape could also play a role as pears were handled more by packers to fit it into the export carton which would provide more contact time between packers' hands and the fruit. The presence of *E. coli*, on pears was most probably a reflection on worker hygiene. According to the literature study the levels at which *E. coli* was found will not present a food safety risk (Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs). The only concern raised by the presence of *E. coli* on pears was that pears should be considered a potential higher risk product in terms of possible microbial contamination and more attention should be given to pears in terms of food safety systems.

The last product exported to Indonesia with test results was Table Grapes, between 2014 and 2019, 364 samples were tested, representing 255 PUC's, covering all SA grape production areas. Grapes were handled the most of all the fruit types, it was picked, transported to the pack house then the bunches were trimmed by hand to remove unsuitable berries and to optimize bunch appearance, and then it was packed into punnets or cartons, despite all the handling and the fact that no post-harvest treatment like washing was allowed no *Salmonella* or significant (≤ 1) *E. coli* was present on Table grapes. The Indonesia samples represent only a small amount of table grape exports. In terms of food safety grapes were considered a high-risk product based on the reason that the whole berry was consumed, and any pathogen on the surface of the grape berry would thus also be ingested. The literature study indicated that due to the cold chain protocols and SO2 sheets included in the grape export cartons, grapes were safe for consumption. Grape packing material carries "a wash before eating" warning as precaution (mostly for residual sulphur dioxide, a known allergen).

Consignment based sampling for microbiological analyses for pathogens, such as the Indonesia samples were not the best and most cost-effective method to determine microbiological risk especially since microbiological contamination was very unpredictable and for 100% certainty you would have to sample 100% of the product which was not possible, especially with something like fresh fruit. The better option would be to do environmental analysis to find potential contamination threats as well as making sure that

GAP's, Pack house food safety systems and worker hygiene was implemented and maintained effectively. One conclusion was that it was very important for the producer and pack house personnel to be very knowledgeable as far as microbiology and pathogens were concerned, as well as responsible and accountable. Producers and pack house managers were responsible to analyse their own microbiological results and if any risks were detected to act on it. If authorities such as DAFF would like to do independent verification of microbiological food safety, it would also be more effective to do environmental analysis especially of water sources and implementation of hygiene practises on production sites as well as in pack houses.

5.2. Discussion of results on Blueberry environmental analysis

The environmental sampling approach represents a risk approach where any possible risks that can possibly contaminate the product, in this case blueberries, with pathogens were investigated. This approach would give a good idea of microbiological risks at the moment of sampling but similar to consignment sampling there was still a high degree of the unpredictable nature of microbiological contamination, and even the environmental study represents a snap shot of the implementation of GAP's and other food safety measures at that specific moment, which could have been compromised by the action of just one poorly trained farm or pack house worker. The environmental microbiological analyses results would give a good picture of implementation and effectiveness of hygiene but was also dependent on the farm and pack house managers knowledge of microbiology and his or her integrity. Producer integrity for regulators such as DAFF and PPECB was critical as it would be impossible for a regulator to do independent analyses of all production environments.

The environmental study results focused on key field activities and equipment in blueberry production. Attention was given to the effectiveness of hygiene of a sample of workers (pickers). The pickers were the only people handling the fruit, blueberries were sorted and packed with mechanical sorting and packing equipment in the pack house. In the environmental study the surfaces that meet the blueberries were also tested, this included picking buckets (the microbiological analysis will reflect the effectiveness of the bucket

cleaning process), transport equipment and water that was used in the field. Microbiological results were mostly aimed at investigating the effectiveness of cleaning and hygiene practices.

Fresh blueberries were also tested by the clients once it reached the destination in the UK, on a consignment. In the consignment both *E. coli* and *Staphylococci* were detected but at limits below codex acceptable maximum levels.

The microbiological results from this environmental study indicated that this blueberry operation maintained high levels of hygiene reducing their microbiological and food safety risk. If results like this was made available to regulators pressure would be placed on producers to implement and maintain food safety integrity.

5.3. Discussion and recommendations based on this study

5.3.1. This study looked at the question, could the SA fruit industry prove to the importing countries that SA fresh produce (fruit) was safe microbiologically?

To date SA fruits were not linked to any food pathogen related outbreak. The Indonesia microbiological results, covering the major export fresh fruit groups and most production areas proofed that SA fruit represent a relative low risk. This does not mean that a potential outbreak was not possible or might have already happened without being positively linked to fruit from SA. This study pointed to the fact that it would be very difficult and costly to do a consignment based microbiological analyses to represent all production units and exported fruit. Results were most likely to follow the trend of the Indonesian results with a high probability that actual microbiological contamination could be missed. Microbiological outbreaks were random and unpredictable and was in most cases not even traceable back to the source. Most likely causes were linked to failure of any one of the many GAP or other food safety measurements. Most of the fruit in the Indonesian results were characterized as low risk, and this was demonstrated by the Indonesian results. The Indonesian results on Table grapes were also an indication that SA table grapes were safe, despite being the most handled fresh product. To maintain this relative safe status of our fresh produce the main

responsibility remains with the producer and pack houses to maintain a very good understanding of their production environment and any possible microbiological risk that could emerge at any point in time, as well as making sure that all farm and pack house workers that work with fresh produce were effectively trained in hygiene. From an independent authority perspective, it was critical to ensure that food safety audits were done thoroughly, independently and with integrity and to scrutinize the audit results as a first line of food safety assurance. DAFF as regulator of food safety should investigate independent environmental microbiological sampling based on risk this could be an important future additional measurement to assure food safety, especially that of the water sources in SA.

5.3.2. This study also looked at the possibility of using the Indonesia microbiological databank with all microbiological analysis from 2014 onwards as a benchmark to assess the current microbiological status of SA fresh fruit.

The Indonesia microbiological analysis represent a very small sample of the total volume of fruit produced and packed for export as well as local market and further to that the fruit drawn for analysis comes from a relatively big pool of fruit being packed from each orchard. To include a fruit that was contaminated with a pathogen in the sample drawn for testing was extremely unlikely, as indicated by these results. The Indonesia results indicated no or insignificant presence of the two key indicator organisms for microbiological contamination *Salmonella* and *E. coli.* These results gave an overall picture that South African fruit up to the pack house level was still relatively safe. The Indonesian approach was not the best option and the better option would be to actively go and search for microbiological contamination risks with environmental studies, including looking at water sources and the actual implementation by farm and pack house worker on good hygiene practices.

5.3.3. The Indonesian microbiological results were also investigated to determine if the Regulatory body DALRRD/PPECB could prevent or eliminate fruit from being exported if microbiological contamination was identified, pro-active outcome.

It was clear from the Indonesian results that it was highly unlikely that we would detect fruit that was contaminated with a pathogen such as *Salmonella* or *E. coli*, which will lead to a rejection of that consignment. A better approach by DALRRD would be to start looking at the production environment and do independent microbiological analysis of water sources, soil, pack house environments and the implementation of hygiene practices to identify high risk production sites and pack houses, similar to the extensive environmental microbiological scans that was undertaken by the USA FDA, especially for high risk products such as melons, berries and avocados. If high risk production sites were identified, fruit from these sites could be tested more regularly to establish improvement. A guide to specific product risk as well as conditions that will increase risk must be developed for South African conditions, so that microbiological risk determination could be approached in a more holistic scientific manner, not depending solely on microbiological analysis.

5.3.4. Building of a database of microbiology linked to exported fruit over time (early warning system) – determining the risk.

The Indonesian microbiological data bank covering 2014 up to now indicates that SA fruit production seems to be relative safe as far as microbiological contamination was concerned, this however does not exclude the possibility that there might be pockets of high risk, and these risks could only be identified by putting in place independent environmental microbiological studies. The integrity of producers was a concern and independent authorities, or regulators must be involved in determining microbiological risk for SA fruit export industry. Studies will have to be repeated over many years in the future to develop early warning systems. One solution would be to access all private microbiological analysis directly from the analyzing laboratory as to prevent producers and pack houses to tamper with results.

5.4. Learned from this study

What micro-organism should we test for, was it enough to look at *E. coli* and *Salmonella*, which was the most likely post-harvest contaminants, what pathogens could contaminate fruit during the growing phase (especially from the soil and irrigation water)?

Salmonella, E. coli and recently *Listeria monocytogenes* were identified as the most critical organism to test for when considering microbiological safety. The presence of these organism will be a good indication that other pathogens might be present as well, and *E. coli* and *Salmonella* would be the best way to identify adherence to the implementation of good agricultural practices and pack house hygiene principle.

Would it be necessary to implement a similar SOP for microbiological contaminants as we currently follow in the MRL SOP, this would determine time, frequency, production unit coverage and type of organisms? (Independent audit samples)

This study indicated that drawing samples on a consignment principle for every PUC and product would give consistent similar results obtained with the Indonesian microbiological results to date. Cost and the very low probability of drawing a contaminated fruit out of a packing run representing a PUC and an orchard indicates that this SOP was not the best option. The focus should rather be on determining how effective producers and pack houses implement their food safety systems during production and packing of fresh fruit.

During orchard inspections it was evident that many orchards, especially those close to human settlements, main roads and farm worker accommodation were very unhygienic in terms of human activity, and the question was, could these unhygienic conditions translate to microbiological contamination of fruit (especially pathogens including viruses)?

The first consideration was type of fruit or fresh product, the Indonesia samples represents fruit such as Citrus, pome fruit and grapes and the environmental study blueberries and from these results it was evident that the risk to contaminate these fruits were very low. One would

need to follow a different approach when working with products such as melons and leafy vegetables where the risk of contamination with soil and irrigation water was extremely high. The key element would always be on how well food safety systems were implemented during production and packing. One would have to take post-harvest treatment into consideration for example citrus fruit was washed and treated with fungicides and waxed (low risk), while products such as pome and stone fruit which was normally not washed and table grapes and strawberries, where no washing was allowed (High risk). The most important aspect when fruit was washed was to make sure the washing water source was potable and during washing processes remains potable. Similarly, water used in pack house cleaning and for personal hygiene must always be potable.

Interventions.

From this study the most effective way to mitigate the microbiological risk associated with fresh fruit would be to first intensify training of producers and pack house managers on all the aspects of fruit microbiology, it was necessary for them to understand the implications if pathogen contamination does occur and what the consequences would be. Training of producers must include the aspects contained in this study such as the type of pathogenic microorganisms, their relation to fresh fruit, sources of contamination, risks associated with different fruit types, and the implications of water and soil contamination. Further to that they need to be made aware of the importance of knowing what was happening in their environment as far as microbiological activity was concerned and follow this up with microbiological environmental scans to be pro-active in terms of possible contamination sources.

Secondly there was a need for independent environmental scans of representing production environments, water sources and pack houses, including independent verification of the implementation of hygiene practices.

From the Indonesia microbiological results, it was clear that consignment based microbiological analysis would not really assist in mitigating the risk effectively. A critical component to the aspect of independence was that auditors responsible for auditing food safety systems must also have a very good understanding of the risk associated with

microbiological contamination and they must make sure that all potential risks such as water sources and worker hygiene were verified.

A third and very important intervention would be consumer education. Consumers also need to know that there was a pathogen risk associated with fresh fruit and vegetables. The FDA (2019) advises consumers to follow important principles to protect them against food poisoning linked to fresh produce. These measures include simple actions such as never buying produce that were bruised, damaged, dirty or suspicious looking (FDA, 2019). If cooling was part of the chain from producer to consumer make sure the cold chain was maintained properly and buy from suppliers with reputable cold chains (FDA, 2019). If packaged, make sure packing material was sealed correctly and avoid fresh produce in damaged packaging (FDA, 2019). Fresh produce that needs to be stored under refrigeration must be transferred to the home fridge as quickly as possible, at home further precautions include proper hand sanitation before and after preparing or eating fresh produce (FDA, 2019). Always wash produce, by rubbing it under running water, before eating or peeling it, so dirt and bacteria present on the surface could be avoided or were not transferred from the knife into the fruit or vegetable (FDA, 2019). If fresh produce was damaged or bruised after you have bought it cut away the damaged or bruised areas before preparing or eating (FDA, 2019). Vegetables closely associated with soil such as potatoes, carrots and a high-risk product such as melons and cucumbers should be washed and scrubbed clean under running water with a vegetable brush, before peeling and cutting, dry produce with a clean cloth or paper towel to further reduce bacteria that may be present (FDA, 2019). Before consuming leafy vegetables such as lettuce and cabbage remove the outermost leaves and wash the remaining leaves under running water or in saltwater (FDA, 2019). Always store fresh produce at the correct temperature and avoid cross contamination with other food such as raw meat (FDA, 2019). People with compromised immune systems should be made aware of the *listeria* risk associated with a product such as melons and should be extra cautious when consuming it.

Microbiological analysis of environmental samples such as that of water sources and the production environment as an important indicator of potential risk was the most important intervention to prevent pathogen outbreaks in the fruit industry, for this to be effective samplers need to be well trained in taking these samples and getting it to laboratories

correctly. This would also be important for instances where it would be necessary to draw fruit samples for microbiological analysis. Independent bodies drawing samples for analysis such as PPECB and DALRRD should make sure that their personnel responsible for this function was properly trained in taking the samples correctly to prevent them from contaminating the samples.

Comparison.

Based on the Indonesian microbiological data the main groups of export fruit namely citrus, table grapes, and pome fruit were microbiologically safe with no rejections for microbiological contamination of fruit. The possibility of contamination without detection or future contamination will always be possible and the Indonesian results indicate how difficult it would be to pinpoint microbiological contamination if it were to happen. Fruit was handled in vast quantities from different production sites and a few contaminated fruits would simply disappear in the greater volume of fruit. It was statically impossible to include that specific fruit in a sample and even if that fruit was included in a sample that was drawn the rest of the fruit in the sample could be free from the pathogen diluting the results to below a risk threshold. This would be the main reasons sampling on a consignment principle would not yield result that was statically useful in predicting microbiological contamination. There was also, a relative high cost to do the analysis with no outcome.

The better alternatives as indicated by this study, was knowledge and acceptance of the responsibility by producers towards food safety in the production environment, continued through the pack house and rest of the cold chain and distribution chains. From a regulatory perspective if would be more effective to do independent environmental studies of microbiological activity and assist producers with indicating areas of risk, such as water sources or poor implementation of hygiene principles.

Outcomes.

Microbiological testing protocol for fresh fruit and vegetables exported from South Africa.

Sampling methodology must focus on independent environmental microbiological analysis of production areas focusing on soil, water and activities that could introduce pathogens into the farm production areas. Independent microbiological sampling must also determine if food safety systems were implemented on farms and in pack houses and especially worker hygiene in fresh fruit production and packing environments, this would assist in preventing potential fresh fruit related food poison out breaks.

Sampling frequency must be based on the risk profile of the fruit and vegetables, production environment, and handling practices. Certain fresh fruit and vegetables for example melons could be considered very high risk and protocols should be developed to establish in which production areas the risk was the highest and how to assist producer in reducing the risk. A key element in preventing microbiological pathogen spread and related food poisoning outbreaks was consumer information and education on the risks associated with fresh fruit and vegetables.

6. Conclusion

Microbiological risk associated with fresh fruit and vegetables remains a reality because pathogens such as *Salmonella, E. coli*, *Listeria* and various other pathogens were common in fresh produce production and handling environments. The most important measure to prevent fresh produce to be contaminated with these pathogens was the implementation of robust food safety systems such as good agricultural practises and HACCP based food safety systems in pack houses. Training for the implementers and participants (orchard workers, pack house workers) in these systems was important to prevent microbiological contamination. All role players must have a good understanding of all factors that could contaminate fresh fruit and vegetables. Environmental microbiology to determine potential contamination risk is of more value than batch sampling. An important factor was that melons, avocados and berries were of higher risk than for example fruit like citrus fruit, and that the association with soil, water and post-harvest interventions plays a key role in microbiological food safety.

From the Indonesia microbiological results used in this study it was clear that routine microbiological testing was not of value and was therefore not recommended. Periodic microbiological testing for specific indicators in the production environment may be more useful for verifying process control and conducting trend analysis. Test for specific pathogens on fruit will only have value when environmental microbiological analyses indicate high potential for contamination or process failure.

Microbiological testing remains an integral part of produce safety programs, but no food safety program could rely solely on microbiological testing, and microbiological testing on a consignment basis will not assist in eliminating the food safety risk due the nature of microbiological contamination. A big problem in South Africa was that microbiological safety was not ultimately verified independently, and a lot of trust was placed in the integrity and ability of food safety auditors and the producers in the implementation of food safety systems.

In terms of microbiological safety, the end consumer must also be well informed on how to mitigate the microbiological food safety risk associated with fresh fruit and vegetables, especially when dealing with high risk products such as melons.

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