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Microbial Performance of Food Safety Control and Assurance Activities in a Fresh Produce Processing Sector Measured Using a Microbial Assessment Scheme and Statistical Modeling

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Abstract

Current approaches such as inspections, audits and sampling for testing cannot detect distribution and dynamics of microbial contamination and food-borne outbreaks linked to fresh produce continue to be reported. A microbial assessment scheme (MAS) and statistical modeling were used to systematically assess the microbial performance of core control and assurance activities in five export fresh produce processing companies. Generalized linear mixed models, and correlated random effects joint models for multivariate clustered data followed by Empirical Bayes estimates enabled analysis of the probability of contamination, across critical sampling locations (CSLs) and factories as random effects. *Salmonella* spp. and *L. monocytogenes* were not detected in final products. However, none of the processors attained the maximum safety level for environmental samples. *E. coli* was detected in 5 out of 6 of the CSLs, including the final product. Amongst the processing environment samples, hands or glove swabs of personnel had higher predicted contamination probabilities with *E. coli*, with 80% of the factories positive at this CSL. End products showed higher predicted probabilities of the lowest level of food safety than raw materials with *E. coli* positive in final products in instances where it was negative in initial products for 60 % of the processors. There was higher probability of contamination with coliforms in water at inlet than the final rinse water. Four out of five (80 %) of the assessed processors had poor to unacceptable counts of *Enterobacteriaceae* on processing surfaces. Personnel, equipment and product related hygiene measures towards improved performance of preventive and intervention measures are recommended.

Key Words: Fresh produce; core control activities; microbial assessment scheme; Empirical Bayes estimates; generalized linear mixed models; correlated random effects joint models.

1 Introduction

Food safety hazards in fresh and fresh-cut vegetables include microorganisms. Vegetables support the growth of micro-organisms, including human pathogenic bacteria. These can be acquired from the production environment (soil, manure, irrigation water) and handling during harvesting, processing (trimming, cutting, peeling, washing, spinning) and packing (WHO, 1998; ICMSF, 2011). Additionally, cutting, slicing and peeling during processing cause tissue damage which releases nutrients therefore facilitating further microbial growth (ICMSF, 2011; Olaimat and Holley, 2012). Given that fresh produce receive minimal or no preparation before consumption, contamination with pathogens along the value chain can pose a serious risk to consumers. It is therefore critical to control bacterial growth for quality and safety of fresh products (ICMSF, 2011).

Producers and processors in the fresh and minimally processed fresh produce chain are required to design and implement effective food safety management systems (FSMS) according to the general principles of food hygiene of the Codex Alimentarius. The most commonly used FSMS standards and quality assurance guidelines include ISO 22000: 2005 and British Retail Consortium (BRC) food safety standard (Jacxsens et al., 2009). These FSMS standards and guidelines combine performance-based approaches such as inspection and sampling for testing. This is meant to evaluate the food safety control system and performance of prerequisite programs such as good hygiene and sanitation programs (Jacxsens et al., 2009). Integrated process-based approaches like FSMSs which combines both control and assurance activities are also applied (Luning and Marcelis, 2009).

However, despite these interventions, bacterial pathogens, viruses and pesticide residues remain a major concern and food-borne outbreaks linked to fresh and minimally processed vegetables and fruits continue to be reported (Van Boxstael et al., 2013). This apparent ineffectiveness of applied FSMS in controlling food safety hazards has been attributed to differences in the translation and implementation of FSMS in the different sectors in the food chain (primary production, processing and trade) (Jin et al., 2008). The disparities in the translation and implementation of FSMS are influenced by technological development, resource availability as well as access to information on standards (FAO, 2007). Situational elements that create risk in decision-making processes and impact design, implementation and operation of FSMS also influence food safety output (Sampers et al., 2010).

Consequently, stakeholders in the agri-food chain such as consumers, sector organizations, regulatory agencies and/or food safety authorities require information on the performance of FSMSs. Such information enables the evaluation of the ability of implemented interventions to improve the microbiological product safety (Luning et al., 2008). The most common method of FSMS evaluation commonly entails checking compliance to specific requirements. However this method does not provide any insight on FSMS performance especially with respect to microbiological hazard levels. Different FSMS standards and guidelines like ISO 22000, BRC and Codex HACCP guidelines recommend system audits and evaluation of critical control points (CCPs) and prerequisite programmes through microbial testing to confirm that selected control measures are effective in eliminating and/or reducing microbial hazards to defined acceptable levels (BRC, 2011; CAC, 2003; Jacxsens et al., 2010). However, this system of verification may not give an indication of the level at which FSMS activities have been translated in a company specific FSMS (Luning and Marcelis, 2009; Luning et al., 2008; Jacxsens et al., 2010). It also

does not provide systematic information on distribution of and variation in microbial contamination (Jacxsens et al., 2009). A previous study on the performance of FSMS control and assurance activities in view of contextual risk revealed weaknesses leading to possibilities of microbial contamination in the fresh produce FSMS (Sawe et al., 2014). The fresh produce exporting companies work with initial materials characterized by high risk of microbial contamination accompanied by partial physical intervention incapable of adequately reducing contamination levels (Sawe et al., 2014). Despite such risk, sampling for microbiological analysis is also variable and some companies do not carry out microbial analysis (Sawe et al., 2014).

A Microbial Assessment Scheme (MAS) tool which allows study of actual microbial performance of core control and assurance activities in an implemented FSMS was developed by Jacxsens et al. (Jacxsens et al., 2009). By tracking proximate indicators such as levels of contamination before and after control points, the impact of particular control measures can be determined (ICMSF, 2006). MAS involves the analysis of selected microbial parameters in certain critical locations on a food establishment over a time interval, usually several months. Microbial safety level profiles are then assigned according to extent to which criteria are met at the critical sampling locations (Jacxsens et al., 2009). This indicates the food safety output of a FSMS and provides an overview of microbial quality, hygiene and safety levels of products and processes.

MAS protocol has been successfully validated and used to highlight aspects requiring improvement in food processing establishments (Jacxsens et al., 2009; Sampers et al., 2010; Oses et al., 2012; Holvoet et al., 2012). However MAS protocol lacks further inferential

statistical treatment of data and such methods will enable further drawing of conclusions from MAS results. It is therefore important to explore the potential of further data modeling for improved inference from MAS.

Generalized linear mixed models (GLMMs) are used for modeling categorical data, accounting for clustering (Aerts et al., 2002; Agresti, 2002; Molenberghs and Verbeke, 2005; Verbeke and Molenberghs, 2000; Fitzmaurice et al., 2009). Correlated random effects joint models, on the other hand, are used for modeling multivariate clustered data (Molenberghs and Verbeke, 2005). A detailed treatise on these models is provided in the supplemental report.

The objective of this study was to assess the actual microbiological performance of control and assurance activities in an export fresh produce processing sector using the MAS protocol and further data modeling. MAS protocol was followed by analysis using generalized linear mixed models and correlated random effects joint models. This enabled to obtain insight on the effectiveness of the FSMS in preventing and/ or reducing microbial contamination or hazards and to recommend aspects towards improvements in fresh produce safety.

2 Materials and methods

2.1 Characterization of firms

Microbial assessment was carried out in five Kenyan fresh produce processing companies whose FSMS had earlier been studied using a FSMS diagnostic instrument (Sawe et al., 2014). The companies process various vegetables including green beans, peas, leafy vegetables (spinach and pakchoi), spring onions, chives, broccoli, herbs and stir-fry mixes (mixed vegetables) destined for export markets (Table 1). They obtain their produce mostly from own farms and from

subcontracted out-growers with GlobalGAP certification. It was further verified using a microbial contamination part of the primary production level of the tool developed by Kirezieva et al. (2013) if conditions at supplying farms predispose fresh produce to microbial contamination. The processing companies studied using MAS are certified to the British Retail Consortium (BRC) food safety management system standard and some of the processors are additionally certified to customer-based standards such as Tesco Nature Source, Woolworths and Marks and Spencer. Operations from sorting to packing were mostly manual. Only P8 had a flume tank with a conveyor belt for produce washing operations. The rest had sets of wash tanks after which the produce was transferred to spinning baskets for drying.

2.2 Food safety output assessment

A modified MAS protocol described by Jacxsens et al. (2009) was used to determine the microbiological food safety output of the FSMS. The protocol involves the selection of: 1) critical sampling locations (CSLs), 2) microbiological parameters or indicators, 3) sampling frequency, 4) sampling and analytical method and 5) criteria for interpretation of results (Jacxsens et al., 2009; Sampers et al., 2010; Oses et al., 2012).

2.2.1 Selection of critical sampling locations

A critical sampling location (CSL) is a location at which contamination, growth, and/ or survival of micro-organisms can occur if the intervention or preventive strategy is not working effectively, or where specific controls and corrective actions have to be carried out to achieve the desired output (Jacxsens et al., 2009).

Product samples included initial materials (CSL 1) and finished products or packaged vegetables (CSL 2). The food contact surface samples included swabs of working tables or chopping boards, conveyer belt, spinning baskets, holding crates and washing troughs (CSL 3), and hand or glove swabs of personnel (CSL 4). Both CSL 3 and 4 are potential sources of cross contamination and provide insight on microbial performance of FSMS preventive measures. Washing water quality was also assessed. The use of water of poor microbial quality can lead to cross-contamination and an increase in microbial load in the end product (Holvoet et al., 2012). Water samples were drawn at inlet to holding tank or washing trough (CSL 5) and at the final rinse water trough (CSL 6). The in-coming water was drawn from the inlet into holding tanks from either borehole or municipal lines. The final rinse water was sampled from the rinsing troughs or flume tanks after addition of chlorine and before introduction of the product. This was aimed at establishing the microbial quality of water used and the effectiveness of the added chlorine in controlling/ eliminating the selected microbial indicators at CSL 6.

2.2.2 Selection of microbiological parameters

Conditions at the growing location and the cultivation system affect the microbial safety of fresh produce (Kirezieva et al., 2013). *Escherichia coli*, *Salmonella spp*. and *Listeria monocytogenes* were therefore selected as food safety indicators. These micro-organisms are indicative of pre-harvest contamination of vegetables either from the production environment, human or animal sources as well as inputs such as manure and irrigation water as well as equipment (ICMSF, 2011; Johnston et al., 2005). *E. coli* and *Enterobacteriaceae* were analyzed as process environment hygiene indicators. Fresh produce safety is dependent on adequate hygiene and sanitation during processing (ICMSF, 2011). *E. coli* and *Staphylococcus aureus* were selected as

indicators of personnel hygiene (Aarnisalo et al., 2006). Only personnel handling the final product or working in the packaging area were swabbed. Coliforms, *E. coli* and *Enterococci*, which are associated with faecal contamination (WHO, 2006), were selected as indicators of water quality.

2.2.3 Sampling frequency

Samples were drawn three times from each firm at different periods between October 2012 and June 2013. For each factory two samples were collected for each of initial product, final product, food contact surfaces and hands/gloves at beginning and end of each working day. One sample of each of incoming and final rinse water was also collected at beginning and end of each working shift per factory. Twenty samples were therefore collected per visit and a total of 60 samples per company were analysed with three hundred samples finally analysed.

2.2.4 Sampling and analytical methods

Sampling protocol and subsequent analysis of microbial parameters was performed using International Organization for Standardization (ISO) methods. Destructive sampling for fresh-cut vegetables was done by collection of 250 g vegetables in sterile stomacher bags. Finished product samples were sampled from the packaged units. Non-destructive sampling was performed for food contact surfaces and hands or gloves by swabbing in accordance with ISO 18593: 2004 horizontal methods. A sterile steel template was used to delineate a sampling area. An area of 50 cm^2 or 25 cm^2 for the food contact surfaces and hands or gloves of the personnel respectively was swabbed using a sterile cotton swab pre-moistened in 10 ml sterile nutrient

broth. All samples were stored and transported to the laboratory in a cool box at ≤ 4°C. Sample preparation was done in accordance with ISO 6887-4: 2003. Test methods for microbial detection and enumeration are shown in Table 2. For enumeration and qualitative detection, 25g of product sample was weighed in a stomacher bag and homogenized for 1 min in 225ml of buffered peptone water (BPW). Swab samples were vortexed for 10 seconds, and the solution incubated in the primary enrichment medium for detection of pathogens or serially diluted for enumeration purposes.

ISO 21528-2:2004 was used for *Enterobacteriaceae* enumeration, which involved pour plate technique using Violet red bile Glucose (VRBG) agar. Colonies of presumptive *Enterobacteriaceae* were then sub cultured on non–selective medium and confirmed, the number was then calculated from the number of confirmed typical colonies per plate. For detection and enumeration of *E. coli*, the method outlined ISO 7521:2005 was used. This involved inoculation of a test sample on VRB plates followed by incubation at 37°C for. Presumptive colonies were confirmed using Kovacs reagent (indole reaction). Detection limits were 0-1 Log cfu per milliliter or gram.

The detection of *Salmonella spp* involved four steps in accordance with ISO 6579:2002. The first step entailed the pre-enrichment of the test portion in buffered peptone water (BPW) at 37°C for 24 hrs. This was followed by selective enrichment of inoculums from the pre-enrichment broth using Rappaport- Vassilladis and Tetrathionate broths at 41°C and 37°C respectively for 24 hrs. After the enrichment steps, solid selective media Xylose Lysine Desoxycholate (XLD) and Brilliant Green Agar (BGA) were used to increase the probability of detecting *Salmonella spp.* These were incubated at 37°C for 18-24 hrs. Presumptive colonies were then subcultured on

Nutrient Agar plates at 37°C for biochemical and serological confirmation. Detection limit was one Log cfu per milliliter or gram.

For detection of *Listeria monocytogenes*, the method described in ISO 11290: 1998 and Amendment 1:2004 was used. This involved the incubation of 25g of sample in Listeria enrichment broth for 24 hours at 30°C followed by isolation and purification using Listeria selective agar (LSA) and Tryptone soya yeast extract (TSYEA) respectively at 30°C for 24-48 hours. Typical colonies were confirmed/tested for haemolysis using sheep blood agar (SBA) and CAMP test.

Staphylococcus aureus was tested in accordance with ISO 6888-3:2003 which involved the inoculation of a test sample on Baird Parker and incubation at 37°C for 24 hours. Both typical and atypical colonies were then subjected to coagulase test using Brain Heart Infusion (BHI) and incubated at 37°C for 24 hrs. Coagulase positive samples were then subjected to biochemical tests using Microbact identification kits. Detection limits were 0-1 Log cfu per milliliter or gram. All analyses included both positive and negative controls and quality control checks as outlined in the respective test methods and laboratory manuals.

Water samples were collected into sterile one-liter bottles and tested using Colilert™ (Idexx Laboratories, Westbrook, Maine) for detection of coliforms and *E. coli*. *Enterococci* were detected using Enterolert™ (Idexx Laboratories, Westbrook, Maine). Samples were incubated for 24 hours at 41°C and 37°C for Enterolert and Colilert respectively. Presence of the microorganisms was indicated by fluorescence (green or blue) under ultra violet (UV) light. Analyses except where specified was done using analytical grade reagents and media (Oxoid) in an ISO 17025 accredited laboratory at the Kenya Bureau of Standards.

2.2.5 Microbiological criterion

Microbiological results for product samples were interpreted against the criteria for ready-to-eat vegetables given in European Commission (EC) Regulation 1441/ 2007 (Anonymous, 2007) and ICMSF (CMSF, 2011). Microbiological guidelines established by the Laboratory of Food Microbiology and Food Preservation, Ghent University (LFMFP-UGhent) were used to evaluate food contact surfaces due to absence of legal criteria (Debevere et al., 2006; Uyttendaelle et al., 2010). Recommendations by Herbert et al. (1990) were used to evaluate hand swabs of personnel. Results for water samples were interpreted against the requirements of Kenya Standard Specification for potable water, part 1, KS 459-1:2007 (Kenya Standards, 2007). Table 2 gives the summary of the CSLs, analyzed parameters, test methods and criteria for interpretation of results.

2.3 Data analyses and interpretation of results

2.3.1 Microbial Assessment Scheme

MAS data was compiled and interpreted for compliance based on criteria given in 2.2.5 above. A food safety level was attributed to each analyzed parameter on a scale of 1 to 3. Level 3 represents a good safety performance, where legal criteria or guidelines are not exceeded. No improvement is required and the current level of the FSMS is adequate to control the respective hazard. Level 2 indicates a moderate safety performance in which improvement is required for a specific control activity of the FSMS. Level 1 represents a poor safety performance where legal criteria or guidelines are exceeded, and improvements are needed on several control activities in the FSMS (Jacxsens et al., 2009). The sum of the food safety levels per CSL gave the MSLP score where the maximum score per CSL was the number of microbial parameters multiplied by

highest performance level three. A score of 0 and 3 was respectively attributed to the presence and absence of a pathogen in a test sample. Table 2 gives the summary of criteria for assigning the food safety levels.

The attributed food safety levels for the microbial parameters were summed up for each CSL to derive Microbiological Safety Level Profiles (MSLPs). This enabled an overview of the FSMS output for each processor at specific CSLs. Microsoft Office Excel 2007 (Microsoft, Redmond, WA) was then used to construct bar graphs and scatter plots to visualize microbial safety level profiles and variations in contamination between the companies across the CSLs (Figure 1).

2.3.2 Statistical Modeling

Statistical analyses were conducted to explore the differences in contamination levels across the critical sampling locations, as well as the factories. Statistical analyses were conducted on the data from *E. coli*, coliforms and *Enterococci* as representative of the tested indicators and because they were tested on at least two critical sampling locations making comparisons among and between critical sampling locations possible.

For *E. coli*, generalized linear mixed models were used. In this case, for CSLs 3, 4, 5, and 6, which represented process environmental samples, this was in the form of logistic regression with random effects. For CSLs 1 and 2, which represented raw materials and final products respectively, this was in the form of the proportional odds model with random effects. Finally, for coliforms and *Enterococci*, the correlated random effects joint model, and the generalized linear mixed models, were used. For detailed analysis of the modeling options available and reasons behind the choice of modeling approaches chosen for this study as most relevant, the reader is referred to the Supplemental report.

281 All the statistical analyses were conducted using SAS software, version 9.4. To illustrate how the
282 statistical models were implemented, sample SAS code with relevant annotation is provided in
283 Appendix B of the Supplemental report.

284

285 **3 Results and discussion**

286 Despite certification of processors/exporters certified to standards such as the International Food
287 Standard (IFS) or GlobalGAP and the BRC food safety standard, reports continue on food borne
288 outbreaks due to contaminated fresh or minimally processed fruits and vegetables (Olaimat and
289 Holley, 2012). An understanding of critical factors influencing microbiological related
290 performance in fresh produce and horticultural production chains would ensure food safety in the
291 short and long term. We illustrate how the impact of established control measures can be
292 assessed by using MAS and statistical modeling.

293 Results from the MAS are shown in Figure 1 and detailed results from exploratory analyses are
294 shown in Supplemental report section 6. *Salmonella spp*. and *L. monocytogenes* were not
295 detected in any of the samples during the sampling period. *E. coli* and *Enterobacteriaceae*
296 showed significant variation, thereby indicating variable performance of FSMS control and
297 assurance activities. FSMS performance was variable and none of the processors attained the
298 maximum score when all CSLs were considered for each processor. This indicates that their
299 FSMS are not operating at optimum and that improvements are therefore needed in control and
300 assurance activities. MAS results can be related to various control activities in the FSMS such as
301 preventive and intervention measures (Luning et al., 2008). Previous results of FSMS activity
302 diagnosis indicate that 77 % of the fresh produce processors operate at basic to moderate levels

(1-2) which might be insufficient in addressing the risk of microbial contamination (Sawe et al., 2014). The MAS results can therefore be related to the actual operation and efficacy of these measures in controlling and/ or reducing selected microbial hazards to acceptable levels (Jacxsens et al., 2009).

The indicator *E. coli* was detected in 5 out of 6 of the CSLs including the final product (CSL 2). *E. coli* was not detected in CSL 6. *E. coli* therefore contributed most to lower food safety levels. From statistical modeling, considering each CSL in each processor as a cluster, the probability of contamination in an average cluster was found to be around 7%, for *E. coli* in process environment samples including food contact surface samples, hand or glove swabs of personnel, incoming and final rinse water. Using the statistical model estimates, the predicted probability of contamination in each cluster for *E. coli* in the process environment samples was computed and the predicted probabilities plotted in Figure 2. The horizontal reference line depicts the contamination probability in an average cluster. For all but one case, the predicted contamination probabilities in clusters involving hands or gloves of personnel were above 7%, with the lowest probability above 7% being 31%, and the highest being 62%. For water samples at the final rinse water trough, only in one case was the contamination probability in a cluster above 7%. On the other hand, the probabilities in all clusters involving P3 were above 7%, whereas in no cluster involving P12 was the probability above 7%. For P3, the proportion of *E. coli* positive samples for any given critical sampling location was at least 0.1667 (16.67%), reaching a high of 100% in the incoming (CSL 5). This was in contrast to P12, where the proportion of *E. coli* positive samples stood at 0% for all the critical sampling locations. For processors 8, 9, and 13, the proportion of *E. coli* positive samples was at least 33%, for at least one of the critical sampling

locations. For the interested reader, technical statistical details related to these results, as well to all the other statistical analysis results discussed here, are available in the Supplemental report.

All the processors attained a food safety level of 3 for *Salmonella spp.* and *L. monocytogenes*. The pathogens were not detected. However, various studies have recommended caution in interpreting results from analysis of pathogens as they may occur at low prevalence in fresh produce (<0.1 to 1 %) leading to low defect rates in food lots (Holvoet et al., 2012; ICMSF, 2011). There was also variation in *Enterobacteriaceae* counts on food contact surface swabs over the sampling period and two processors did not meet criteria in all the analyzed samples. Performance by two other processors was poor and average while one processor met the criteria for this parameter.

Based on statistical analyses, where each CSL in each processor was again considered as a cluster, the probability of the lowest, second, and highest level of food safety, was respectively found to be 28%, 17%, and 55%, for initial materials (CSL 1) and final products (packaged vegetables) (CSL 2). Based on the model estimates, the predicted probability of each level of food safety, in each cluster, was also computed. These probabilities are plotted in Figure 3, with the horizontal reference line depicting the probability in average clusters. With regard to the lowest level of food safety, only in one cluster involving raw materials (CSL 1) was the estimated probability in an average cluster (28%) exceeded, at 39% (Fig 3a). For the second level of food safety, the probabilities in almost all clusters were very close to the probability in an average cluster (17%) (Fig 3b). At the highest food safety performance level, in 4 out of the 5 clusters involving raw materials (CSL 1), the predicted probabilities were at or above the probability in an average cluster (Fig 3c). An average food safety level of 2 was attributed from

MAS protocol for all the processors at this CSL. These results indicate that preventive measures such as good agricultural practices, farm hygiene and personnel hygiene are able to limit contamination (ICMSF, 2011). All the processors sourced their initial materials from Global GAP certified farms with certain set minimum food safety assurance activity requirements. Such food safety assurance activities are a prerequisite for initial materials of good microbial quality (Jacxsens et al., 2009). As indicated from the our verification of conditions at the Global GAP certified supplying farms, production conditions were likely not to pose risk of microbial contamination of raw materials.

More rigorous FSMS controls are required to ensure that the microbial quality of the end product meets food safety criteria whenever raw materials are contaminated (Jacxsens et al., 2009). Poor performance at this level may put a strain on the FSMS controls at subsequent processing stages (Luning et al., 2008). Microbiological performance of initial materials therefore provides information on potential safety risks associated with raw materials which in turn influences the rigorousness of FSMS interventions (Kirezieva et al., 2013; Luning et al., 2008).

Majority (60 %) of processors performed poorly (assigned food safety level of 1) with respect to *E. coli* in the final products at CSL 2 (Fig 1b). A comparatively high number of samples were at the lowest food safety level for all, except one processor at this CSL (Supplemental Report). In 3 out of the 5 clusters involving final products, the probability of the lowest food safety level was noticeably above that in an average cluster. These predicted contamination probabilities were above this average percentage, in particular, at 53%. The poor performance is indicative of either inadequate decontamination processes or contamination from the processing environment, equipment or human handling. Lack of sanitizer efficiency in removing or killing pathogens on

raw fruits and vegetables has also been attributed to structures and tissues that may harbor pathogens (Fieuws and Verbeke, 2004). The microbial quality of end products gives an indication of the effectiveness of applied interventions in preventing and/or reducing microbial hazards to acceptable levels and the overall performance of the FSMS (Luning and Marcelis, 2007). Such intervention measures include hygiene and sanitation, and decontamination processes (Jacxsens et al., 2009). The most common method of decontamination among the processors was successive washing followed by rinsing with chilled (4-8°C) chlorinated water (40- 80 ppm of chlorine) for an average of 5 minutes. However, pH and concentration of chlorine was not checked to ensure maintenance of concentrations effective in decontaminating the product. This can be attributed to low level of monitoring systems and deficiencies in standards and tolerances with respect to product and process monitoring (Sawe et al., 2014). For chlorine to be effective, a combination of its concentration, pH and contact time with product is important (WHO, 1998). Microbial cells present in the initial product might therefore persist to the end product when active components and conditions necessary for their inactivation are not monitored. The risk posed by low level contamination can be enhanced by cross- contamination during washing, surface moisture and temperature variation (Danyluk and Schaffner, 2011). Factors such as hydrophobicity of plant surfaces and biofilm formation by bacterial cells have also been shown to limit the effectiveness of post-harvest washing in reducing the microbial load (Frank, 2001). In addition, water flumes used during processing can spread initial spot contamination. Johnston et al. (2005) reported that the level of coliforms in parsley and cilantro increased after washing especially at the rinsing step. Furthermore, contamination can originate or increase during packing (Johnston et al., 2005). This might therefore explain the presence of

391 *E. coli* in the final product where it was not detected in the initial products from two processors
392 (P9 and P12).

393 The product contact surfaces (CSL 3) including produce holding crates, bowls, spinning baskets
394 and their liners, conveyor belts, chopping boards and work tables had total MSLP of 6. None of
395 the processors achieved the maximum level for this CSL (Fig. 1c). The results for *E. coli* and
396 *Enterobacteriaceae* at the CSL were variable over the sampling period. One processor (P3) had
397 the lowest MSLP of 2 due to the presence of *Enterobacteriaceae* counts above the maximum
398 limit. *E. coli* was detected in food contact surface swabs of two processors. This was mainly in
399 crates used to hold the product after spin-drying prior to packaging. The crates are made from
400 plastic and its construction may facilitate adherence of micro-organisms due to the presence of
401 perforations which may make cleaning difficult. Containers coming into contact with products
402 should be designed and constructed in a way that makes them easy to clean, disinfect and
403 maintain to avoid contamination of product (CAC, 2003). On the other hand, 80 % of the
404 assessed processors had poor to unacceptable performance for the indicator *Enterobacteriaceae*
405 (0 to 3.2 log CFU/cm^2 against a maximum of log 2.5 CFU/cm^2 as per the guidelines). This was
406 despite hygienic design of equipment and facilities being categorized as advanced in a previous
407 study (Sawe et al., 2014). Cleaning and sanitation procedures were therefore not effective in
408 reducing microbial contamination to acceptable levels. Microorganisms may also adhere to food
409 contact surfaces in form of biofilms even after sanitation (Frank, 2001). The poor performance at
410 CSL 3 can therefore compromise food safety through cross contamination. The processors
411 undertook verification after cleaning and sanitation at intervals. However, the frequency of
412 verification was not defined and may therefore not be satisfactory in determining the
413 effectiveness of the programmes. Sanitation programmes tailored and supported with appropriate

414 instructions and verified for effectiveness in eliminating hazards should be implemented. These
415 programmes should be modified when results of verification show deviations from specifications
416 (CAC, 2003; Luning et al., 2008).

417 The highest proportion of *E. coli* positive results was observed in personnel hand swabs (CSL 4),
418 with 33% of the samples being positive. MSLPs of 4-5 out of a maximum of 6 were recorded
419 (Fig 1d). *S. aureus* was detected in one personnel hand swab from one processor (P12) on one
420 sampling occasion hence a food safety level of 2. *E. coli* was detected in hand swabs of 80 % of
421 the assessed processors hence food safety levels of 1-2 for the indicator. The poor performance at
422 the CSL with respect to *E. coli* might have resulted from contact with environment which
423 contaminates hands with transient flora such as *E. coli* and *Salmonella spp.* (Dijk et al., 2007).
424 The CSL is a critical control point in the FSMS as most operations are manual and inadequate
425 compliance to hygienic practices may compromise the safety of end products. Personnel hygiene
426 is important in prevention of direct and indirect contamination of food because hands can
427 contaminate food through skin associated flora such as *Staphylococci* (Aarnisalo et al., 2006;
428 Dijk et al., 2007). However, no relationship was established between the detection of *E. coli* on
429 personnel hands and in the final product. For example, *E. coli* was not detected in any personnel
430 swabs of P12 but the indicator was detected in the final product on two occasions. This means
431 that contamination of end product with *E. coli* may have originated from other sources in the
432 processing environment. Both CSL 3 and 4 are potential sources of cross contamination and
433 provide insight on microbial performance of FSMS preventive measures. Such preventive
434 measures include hygienic design of equipment and facilities, specificity of cleaning and
435 sanitation and compliance to hygiene requirements by personnel (Jacxsens et al., 2010).

Water quality was assessed in CSL 5 (incoming water) and 6 (final rinse water), each with a total MSLP of 9. *Enterococci* and coliforms were tested simultaneously at CSL 5 and 6. The 30 samples which tested negative for coliforms, also tested negative for *Enterococci*, while 8 samples which tested positive for coliforms, also tested positive for *Enterococci*. Incoming water had the second highest *E. coli* contamination with 27% samples testing positive. The percentage of positive samples for incoming water was 67% in two factories, and 100% in one factory. Based on statistical modeling results, where each CSL in each processor was again considered as a cluster, the probabilities of contamination in an average cluster, with respect to coliforms and *Enterococci* respectively, were found to be 0.061%, and 33.60%. Contamination probabilities in the clusters were also computed, using the model estimates, and plotted in Figure 4. While in most clusters the probabilities for coliform contamination were around the estimated probability in an average cluster (0.061%), the probabilities in 3 out of the 5 clusters involving water at inlet were conspicuously way above, at 65%, with one cluster at 98%. For *Enterococci*, the probabilities in most of the clusters were close to or below the probability in an average cluster (33.60%), with the predicted contamination probabilities in two clusters involving incoming water being above 90% (Fig 4b).

Washing is a partial intervention step in fresh produce processing. Washing is a critical step in reducing microbiological contamination which also removes some of the cell exudates which support microbial growth at cut surfaces (CAC, 2003; Harris et al., 2003). However, washing has been identified as a potential step through which microbial hazards can be introduced and especially if microbial quality of the water is unsatisfactory (Holvoet et al., 2012). Washing of fresh cut produce therefore requires use of potable water in order to prevent the transfer of

458 contamination from water to the produce. Processor P3 had a food safety level of 0 for CSL 5
459 due to the detection of coliforms, *E. coli* and *Enterococci* in their incoming water (Fig 1e). P9
460 also had a poor performance at the CSL due to the presence of coliforms and *E. coli* in the
461 incoming water. Processor P12 had a MSLP of 7 for the CSL while P8 attained a MSLP of 8.
462 *Enterococci* was detected in the incoming water on all samples drawn from P13, hence a MSLP
463 of 6. This might be due to contamination or the presence of biofilms in the piping system
464 (Hallam et al., 2001). Processors P3 and P13 sourced their water from boreholes while the rest
465 used municipal water. There was a better output at CSL 6 (Fig 1f) because all the firms treated
466 their water with chlorine prior to using it to rinse the product. In all clusters involving final rinse
467 water, the predicted contamination probabilities were extremely small (only 0.045%). Two
468 processors attained the maximum MSLP of 9 at CSL 6. However, *Enterococci* were still detected
469 in the final rinse water from three processors. The predicted contamination probability in one
470 cluster reached 63% (Fig 4). This may either be due to poor cleaning of flume tanks or
471 ineffective water treatment.

472 There was variation in contamination with *E. coli*. This may be attributed to inadequate cleaning
473 and sanitation as well as cross contamination. *Enterobacteriaceae* counts at CSL 3 ranged from
474 poor to unsatisfactory (food safety level of 0 or 1) in 80 % of the firms. However, processor P13
475 had a good food safety compared to the other companies. Its final product met the criteria
476 throughout the sampling period and cleaning and sanitation was effective with either absence or
477 low variation in *E. coli* contamination when present. *Enterobacteriaceae* counts were also
478 within the guidelines. *E. coli* was detected on only one out of twelve personnel swabs. The
479 FSMS control and assurance activities for P13 therefore seem effective in controlling microbial
480 hazards though water quality monitoring needs to be enhanced to ensure compliance with

specifications. Processor P3 had the least performing FSMS as depicted by the MAS results (Fig. 1). Food safety levels at some CSLs for processor P3 indicated unsatisfactory performance of control activities and prerequisite programs in preventing or controlling microbial hazards to acceptable levels. This poor output was mainly contributed by CSL 3 (food contact surfaces) and CSL 5 (incoming water). Equipment and facilities hygiene is therefore crucial in prevention of cross-contamination throughout the processing environment. Practices such as insufficient washing of wash or flume tanks have been found to increase the potential to transfer *E. coli* contamination to the end product (ICMSF, 2011; Johnston et al., 2005). Hygienic design of equipment and facilities must be supported by adequate cleaning and sanitation programmes with performance tests done on a regular basis (Luning et al., 2008).

Verification of cleaning and sanitation should therefore be improved for all the processors. Effectiveness of cleaning requires re-validation in order to improve general hygiene and reduce the possibility of cross-contamination. This will facilitate development of more effective sanitation programmes adapted for various production zones that will counter risk of cross contamination. Cleaning and sanitation programmes should be based on analyzed historical data for each company, and cleaning and sanitation tailored for different equipment and facilities. In addition, the frequency of cleaning and sanitation should be based on results of verification activities.

Finally, from a statistical point of view, in the design of future studies, it would be interesting to combine aspects of the MAS protocol with aspects of survey sampling methodology (Groves et al., 2004; Milanzi et al., 2015). The MAS protocol could be utilized in defining the critical sampling locations, with survey sampling principles being used to specify a survey sampling

503 design, and to calculate the number of factories and the number of samples, at each critical
504 sampling location in each factory, that would be needed to achieve various statistical analysis
505 objectives. A possibility in terms of the survey sampling design would be to consider the
506 factories as clusters, and the critical sampling locations as strata; the above-mentioned
507 calculations could then be conducted. Evidently, the "intra-factory" correlation would be an
508 important input. This correlation could be estimated in the framework of a beta-binomial model
509 (Aerts et al., 2002; Faes et al., 2009; Fitzmaurice et al., 2009; Molenberghs and Verbeke, 2005).

510 **4 Conclusion**

511 We used a microbial assessment scheme together with statistical modeling to provide insight on
512 microbial performance of control and assurance activities in fresh produce processing sector.
513 Higher coliforms contamination probabilities in water at inlet in comparison to final rinse water
514 shows effectiveness of water treatment prior to use in processing. However, the presence of *E.*
515 *coli* in the end product at higher probabilities than raw materials indicated ineffectiveness of
516 FSMS control measures which may be due to inadequate monitoring at critical steps. Presence of
517 *E. coli* in final products where it was not detected in the initial product or food contact surfaces
518 indicates cross-contamination. There is therefore possibility of spread in spot contamination
519 during washing, poor cleaning and sanitation (preventive measures) of flume tanks, inadequate
520 intervention processes (decontamination) and inadequate monitoring systems.

521 Better performance of control and assurance activities will be contingent upon improvements in
522 preventive measures fresh produce tailored cleaning and sanitation programs, personnel hygiene
523 and hygienic design of equipment and facilities. Re-evaluation of intervention measures coupled
524 with adequate monitoring methods are also necessary to assure food safety. Future research

direction is suggested where the MAS protocol is utilized in defining the critical sampling locations, with survey sampling principles being used to specify a survey sampling design, and to calculate the number of factories and the number of samples, at each critical sampling location in each factory, that would be needed to achieve various statistical analysis objectives.

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656

Figure Legends

Figure 1. Microbial safety level profiles at critical sampling locations for fresh produce processing firms (a) CSL 1, initial products; (b) CSL 2, final products; (c) CSL 3, product contact surfaces; (d) CSL 4, personnel hands and/or gloves; (e) CSL 5, incoming water; (f) CSL 6, final rinse water. P3, P8, P9, P12, P13- processors 3, 8, 9, 12 and 13.

Figure 2 Predicted *E. coli* contamination probabilities in processor-by-critical sampling location clusters for process environment samples including food contact surface samples, hand or glove swabs of personnel, water at inlet and at the final rinse water trough

Probabilities computed from estimates of a logistic regression model with random effects. Horizontal line represents the contamination probability in an average cluster, calculated by setting the random effect in the model to 0 (the mean of the random effects distribution).

Figure 3 Probability of (a) lowest food safety performance level, (b) food safety performance level 2, (c) highest food safety performance level, and (d) each of the three food safety performance levels, together, in each processor-by-critical sampling location combination for initial materials and final products

Horizontal reference lines depict the probability in an average cluster calculated by setting the random effect in the model to 0 (the mean of the random effects distribution).

676 Figure 4 Probability of contamination of water at inlet and final rinse water with in each
677 processor-by-critical sampling location combination with (a) Coliforms and (b) *Enterococci*

678 Probabilities computed from estimates of logistic regression models with random effects.
679 Horizontal lines represent the probability in an average cluster, calculated by setting the random
680 effect in the model to 0 (the mean of the random effects distribution).

681

682 Tables

683 Table 1. Characteristics of fresh produce processors assessed for microbial performance of safety
684 management systems

	Processor				
Characteristic	**P3**	**P8**	**P9**	**P12**	**P13**
Total No. of employees	50- 249	50-249	50-249	50-249	50-249
No. in QA Dept	20	33	35	50	120
Products	F, V	V, H	F, V	V	V, H
QA standard certified	BRC	BRC, CBS	BRC, CBS	BRC	BRC, CBS
Tonnage exported per annum	3000	7800	5000	7000	7000

F- fruits; V- vegetables; H- Herbs; BRC- British Retail Consortium; CBS- Customer-based Standards (Tesco Nature Source (TNS), Woolworths and Marks & Spencer); QA- Quality Assurance.

688 Table 2. Critical sampling locations, analyzed microbiological parameters, test methods and criteria for interpretation of results on
689 microbial performance of safety management systems in fresh produce

690

CSL	Description	Parameters	Test method	Limits	Reference
1	Initial products	*E. coli*	ISO 7521: 2005	$m<10^2$, $M<10^3$	EC 1441/2007
		Salmonella spp	ISO 6579:2002	Absent in 25 g	EC 1441/2007
2	Final product	*E. coli*	ISO 7521: 2005	$m<10^1$, $M<10^2$	EC 1441/2007
		Salmonella spp	ISO 6579:2002	Absent in 25 g	EC 1441/2007
		L. monocytogenes	ISO 11290-2:1998/Amendment	Absent in 25 g	EC 1441/2007

			1:2004		
3	Food contact surfaces	*E. coli*	ISO 7521: 2005	≤0.7 log CFU/50 cm2	[a]LFMFP, UGhent
		Enterobacteriaceae	ISO 21528-2:2004	Good, ≤ 1; Average, ≤ 1.8; Bad, ≤ 2.5; Intolerable, > 2.5 log colony forming units	
4	Hand/glove swabs	*S. aureus*	ISO 6888-3:2003	≤1.7 log CFU/25 cm^2 (below detection limit)	[a]LFMFP, UGhent

		E. coli	ISO 7521: 2005	≤0.7 log CFU/25 cm^2 (below detection limit)	
5	Water at inlet	Coliforms	ISO 9308-1:2000	Absent/100ml	KS 459
		E. coli	ISO 9308-1:2000	Absent/100ml	
		Enterococci	ISO 7899-2:2000	Absent/100ml	
6	Final rinse water	Coliforms	ISO 9308-1:2000	Absent/100ml	KS 459
		E. coli	ISO 9308-1:2000	Absent/100ml	
		Enterococci	ISO 7899-2:2000	Absent/100ml	

691 [a]LFMFP- Laboratory of Food Microbiology and Food Preservation, Ghent University, Belgium; EC- European Commission
692 Regulation; KS- Kenya Standard, m is maximum level of bacteria per test volume considered acceptable; M is maximum level of
693 bacteria per test volume considered marginally acceptable (values at or above M are unacceptable).

694 Table 3. Criteria for attribution of food safety level scores

Food safety level	[a]Criteria
3	$R \leq m$ or organism absent in x grams, milliliters or 50 cm^2 or 25 cm^2
2	$m < R < M$
1	$R \geq M$

695 [a] R, results obtained from analysis; m, maximum level of bacteria per test volume considered
696 acceptable; M, maximum level of bacteria per test volume considered marginally acceptable
697 (values at or above M are unacceptable)