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

22 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 23 continue to be reported. A microbial assessment scheme (MAS) and statistical modeling were 24 25 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 26 correlated random effects joint models for multivariate clustered data followed by Empirical 27 Bayes estimates enabled analysis of the probability of contamination, across critical sampling 28 locations (CSLs) and factories as random effects. Salmonella spp. and L. monocytogenes were 29 30 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 31 32 final product. Amongst the processing environment samples, hands or glove swabs of personnel 33 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 34 safety than raw materials with E. coli positive in final products in instances where it was 35 negative in initial products for 60 % of the processors. There was higher probability of 36 contamination with coliforms in water at inlet than the final rinse water. Four out of five (80 %) 37 38 of the assessed processors had poor to unacceptable counts of *Enterobacteriaceae* on processing surfaces. Personnel, equipment and product related hygiene measures towards improved 39 40 performance of preventive and intervention measures are recommended.

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

43 **1 Introduction**

44 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 45 acquired from the production environment (soil, manure, irrigation water) and handling during 46 harvesting, processing (trimming, cutting, peeling, washing, spinning) and packing (WHO, 1998; 47 ICMSF, 2011). Additionally, cutting, slicing and peeling during processing cause tissue damage 48 49 which releases nutrients therefore facilitating further microbial growth (ICMSF, 2011; Olaimat and Holley, 2012). Given that fresh produce receive minimal or no preparation before 50 consumption, contamination with pathogens along the value chain can pose a serious risk to 51 52 consumers. It is therefore critical to control bacterial growth for quality and safety of fresh products (ICMSF, 2011). 53

Producers and processors in the fresh and minimally processed fresh produce chain are required 54 55 to design and implement effective food safety management systems (FSMS) according to the 56 general principles of food hygiene of the Codex Alimentarius. The most commonly used FSMS 57 standards and quality assurance guidelines include ISO 22000: 2005 and British Retail 58 Consortium (BRC) food safety standard (Jacxsens et al., 2009). These FSMS standards and 59 guidelines combine performance-based approaches such as inspection and sampling for testing. 60 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 61 62 process-based approaches like FSMSs which combines both control and assurance activities are also applied (Luning and Marcelis, 2009). 63

64 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 65 and fruits continue to be reported (Van Boxstael et al., 2013). This apparent ineffectiveness of 66 applied FSMS in controlling food safety hazards has been attributed to differences in the 67 translation and implementation of FSMS in the different sectors in the food chain (primary 68 69 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 70 well as access to information on standards (FAO, 2007). Situational elements that create risk in 71 72 decision-making processes and impact design, implementation and operation of FSMS also influence food safety output (Sampers et al., 2010). 73 Consequently, stakeholders in the agri-food chain such as consumers, sector organizations, 74 regulatory agencies and/or food safety authorities require information on the performance of 75 76 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 77 FSMS evaluation commonly entails checking compliance to specific requirements. However this 78 method does not provide any insight on FSMS performance especially with respect to 79 microbiological hazard levels. Different FSMS standards and guidelines like ISO 22000, BRC 80 81 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 82 83 measures are effective in eliminating and/or reducing microbial hazards to defined acceptable 84 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 85 86 specific FSMS (Luning and Marcelis, 2009; Luning et al., 2008; Jacxsens et al., 2010). It also

87 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 88 assurance activities in view of contextual risk revealed weaknesses leading to possibilities of 89 90 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 91 92 contamination accompanied by partial physical intervention incapable of adequately reducing contamination levels (Sawe et al., 2014). Despite such risk, sampling for microbiological 93 analysis is also variable and some companies do not carry out microbial analysis (Sawe et al., 94 95 2014).

96 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 97 Jacxsens et al. (Jacxsens et al., 2009). By tracking proximate indicators such as levels of 98 99 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 100 certain critical locations on a food establishment over a time interval, usually several months. 101 Microbial safety level profiles are then assigned according to extent to which criteria are met at 102 the critical sampling locations (Jacxsens et al., 2009). This indicates the food safety output of a 103 FSMS and provides an overview of microbial quality, hygiene and safety levels of products and 104 105 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

109	statistical treatment of data and such methods will enable further drawing of conclusions from
110	MAS results. It is therefore important to explore the potential of further data modeling for
111	improved inference from MAS.
112	Generalized linear mixed models (GLMMs) are used for modeling categorical data, accounting for
113	clustering (Aerts et al., 2002; Agresti, 2002; Molenberghs and Verbeke, 2005; Verbeke and
114	Molenberghs, 2000; Fitzmaurice et al., 2009). Correlated random effects joint models, on the
115	other hand, are used for modeling multivariate clustered data (Molenberghs and Verbeke, 2005).
116	A detailed treatise on these models is provided in the supplemental report.
117	The objective of this study was to assess the actual microbiological performance of control and
118	assurance activities in an export fresh produce processing sector using the MAS protocol and
119	further data modeling. MAS protocol was followed by analysis using generalized linear mixed
120	models and correlated random effects joint models. This enabled to obtain insight on the
121	effectiveness of the FSMS in preventing and/ or reducing microbial contamination or hazards
122	and to recommend aspects towards improvements in fresh produce safety.

2 Materials and methods 123

124 **2.1 Characterization of firms**

Microbial assessment was carried out in five Kenyan fresh produce processing companies whose 125 FSMS had earlier been studied using a FSMS diagnostic instrument (Sawe et al., 2014). The 126 companies process various vegetables including green beans, peas, leafy vegetables (spinach and 127 pakchoi), spring onions, chives, broccoli, herbs and stir-fry mixes (mixed vegetables) destined 128 for export markets (Table 1). They obtain their produce mostly from own farms and from 129

130 subcontracted out-growers with GlobalGAP certification. It was further verified using a 131 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 132 133 contamination. The processing companies studied using MAS are certified to the British Retail 134 Consortium (BRC) food safety management system standard and some of the processors are 135 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 136 flume tank with a conveyor belt for produce washing operations. The rest had sets of wash tanks 137 138 after which the produce was transferred to spinning baskets for drying.

2.2 Food safety output assessment 139

A modified MAS protocol described by Jacxsens et al. (2009) was used to determine the 140

microbiological food safety output of the FSMS. The protocol involves the selection of: 1) 141

critical sampling locations (CSLs), 2) microbiological parameters or indicators, 3) sampling 142

143 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). 144

145 2.2.1 Selection of critical sampling locations

146 A critical sampling location (CSL) is a location at which contamination, growth, and/ or survival 147

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 148

149 desired output (Jacxsens et al., 2009). 150 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, 151 conveyer belt, spinning baskets, holding crates and washing troughs (CSL 3), and hand or glove 152 swabs of personnel (CSL 4). Both CSL 3 and 4 are potential sources of cross contamination and 153 provide insight on microbial performance of FSMS preventive measures. Washing water quality 154 was also assessed. The use of water of poor microbial quality can lead to cross-contamination 155 and an increase in microbial load in the end product (Holvoet et al., 2012). Water samples were 156 drawn at inlet to holding tank or washing trough (CSL 5) and at the final rinse water trough (CSL 157 158 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 159 addition of chlorine and before introduction of the product. This was aimed at establishing the 160 161 microbial quality of water used and the effectiveness of the added chlorine in controlling/ eliminating the selected microbial indicators at CSL 6. 162

163 2.2.2

2.2.2 Selection of microbiological parameters

Conditions at the growing location and the cultivation system affect the microbial safety of fresh 164 produce (Kirezieva et al., 2013). Escherichia coli, Salmonella spp. and Listeria monocytogenes 165 166 were therefore selected as food safety indicators. These micro-organisms are indicative of preharvest contamination of vegetables either from the production environment, human or animal 167 sources as well as inputs such as manure and irrigation water as well as equipment (ICMSF, 168 2011; Johnston et al., 2005). E. coli and Enterobacteriaceae were analyzed as process 169 environment hygiene indicators. Fresh produce safety is dependent on adequate hygiene and 170 sanitation during processing (ICMSF, 2011). E. coli and Staphylococcus aureus were selected as 171

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.

176 **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.

183

184 **2.2.4 Sampling and analytical methods**

Sampling protocol and subsequent analysis of microbial parameters was performed using 185 International Organization for Standardization (ISO) methods. Destructive sampling for fresh-cut 186 vegetables was done by collection of 250 g vegetables in sterile stomacher bags. Finished 187 product samples were sampled from the packaged units. Non-destructive sampling was 188 performed for food contact surfaces and hands or gloves by swabbing in accordance with ISO 189 18593: 2004 horizontal methods. A sterile steel template was used to delineate a sampling area. 190 An area of 50 cm^2 or 25 cm^2 for the food contact surfaces and hands or gloves of the personnel 191 respectively was swabbed using a sterile cotton swab pre-moistened in 10 ml sterile nutrient 192

broth. All samples were stored and transported to the laboratory in a cool box at \leq 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.

200 ISO 21528-2:2004 was used for Enterobacteriaceae enumeration, which involved pour plate technique using Violet red bile Glucose (VRBG) agar. Colonies of presumptive 201 202 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 203 enumeration of E. coli, the method outlined ISO 7521:2005 was used. This involved inoculation 204 205 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 206 milliliter or gram. 207

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.

230 Water samples were collected into sterile one-liter bottles and tested using ColilertTM (Idexx

231 Laboratories, Westbrook, Maine) for detection of coliforms and E. coli. Enterococci were

detected using EnterolertTM (Idexx Laboratories, Westbrook, Maine). Samples were incubated

for 24 hours at 41°C and 37°C for Enterolert and Colilert respectively. Presence of the

234 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.

237 2.2.5 Microbiological criterion

238 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

- 240 ICMSF (CMSF, 2011). Microbiological guidelines established by the Laboratory of Food
- 241 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.,
- 243 2010). Recommendations by Herbert et al. (1990) were used to evaluate hand swabs of
- 244 personnel. Results for water samples were interpreted against the requirements of Kenya
- 245 Standard Specification for potable water, part 1, KS 459-1:2007 (Kenya Standards, 2007). Table
- 246 2 gives the summary of the CSLs, analyzed parameters, test methods and criteria for
- 247 interpretation of results.
- 248 **2.3 Data analyses and interpretation of results**

249 2.3.1 Microbial Assessment Scheme

MAS data was compiled and interpreted for compliance based on criteria given in 2.2.5 above. A 250 food safety level was attributed to each analyzed parameter on a scale of 1 to 3. Level 3 251 252 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 253 254 hazard. Level 2 indicates a moderate safety performance in which improvement is required for a 255 specific control activity of the FSMS. Level 1 represents a poor safety performance where legal 256 criteria or guidelines are exceeded, and improvements are needed on several control activities in 257 the FSMS (Jacxsens et al., 2009). The sum of the food safety levels per CSL gave the MSLP 258 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).

267 **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, 273 which represented process environmental samples, this was in the form of logistic regression 274 275 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, 276 for coliforms and *Enterococci*, the correlated random effects joint model, and the generalized 277 linear mixed models, were used. For detailed analysis of the modeling options available and 278 reasons behind the choice of modeling approaches chosen for this study as most relevant, the 279 280 reader is referred to the Supplemental report.

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

284

285 **3 Results and discussion**

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

Results from the MAS are shown in Figure 1 and detailed results from exploratory analyses are 293 shown in Supplemental report section 6. Salmonella spp. and L. monocytogenes were not 294 detected in any of the samples during the sampling period. E. coli and Enterobacteriaceae 295 showed significant variation, thereby indicating variable performance of FSMS control and 296 297 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 assurance activities. MAS results can be related to various control activities in the FSMS such as 300 preventive and intervention measures (Luning et al., 2008). Previous results of FSMS activity 301 diagnosis indicate that 77 % of the fresh produce processors operate at basic to moderate levels 302

303 (1-2) which might be insufficient in addressing the risk of microbial contamination (Sawe et al.,

304 2014). The MAS results can therefore be related to the actual operation and efficacy of these

305 measures in controlling and/ or reducing selected microbial hazards to acceptable levels

306 (Jacxsens et al., 2009).

The indicator *E. coli* was detected in 5 out of 6 of the CSLs including the final product (CSL 2). 307 E. coli was not detected in CSL 6. E. coli therefore contributed most to lower food safety levels. 308 From statistical modeling, considering each CSL in each processor as a cluster, the probability of 309 contamination in an average cluster was found to be around 7%, for E. coli in process 310 environment samples including food contact surface samples, hand or glove swabs of personnel, 311 312 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 313 the predicted probabilities plotted in Figure 2. The horizontal reference line depicts the 314 315 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 316 probability above 7% being 31%, and the highest being 62%. For water samples at the final rinse 317 water trough, only in one case was the contamination probability in a cluster above 7%. On the 318 other hand, the probabilities in all clusters involving P3 were above 7%, whereas in no cluster 319 involving P12 was the probability above 7%. For P3, the proportion of E. coli positive samples 320 for any given critical sampling location was at least 0.1667 (16.67%), reaching a high of 100% in 321 322 the incoming (CSL 5). This was in contrast to P12, where the proportion of *E. coli* positive 323 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 324

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

327 All the processors attained a food safety level of 3 for *Salmonella spp.* and *L. monocytogenes*.

328 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,

331 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 criteriafor this parameter.

335 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 336 found to be 28%, 17%, and 55%, for initial materials (CSL 1) and final products (packaged 337 338 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 339 the horizontal reference line depicting the probability in average clusters. With regard to the 340 341 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 342 level of food safety, the probabilities in almost all clusters were very close to the probability in 343 an average cluster (17%) (Fig 3b). At the highest food safety performance level, in 4 out of the 5 344 clusters involving raw materials (CSL 1), the predicted probabilities were at or above the 345 probability in an average cluster (Fig 3c). An average food safety level of 2 was attributed from 346

347 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 348 contamination (ICMSF, 2011). All the processors sourced their initial materials from Global 349 350 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 351 (Jacxsens et al., 2009). As indicated from the our verification of conditions at the Global GAP 352 certified supplying farms, production conditions were likely not to pose risk of microbial 353 contamination of raw materials. 354

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 361 E. coli in the final products at CSL 2 (Fig 1b). A comparatively high number of samples were at 362 363 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 364 noticeably above that in an average cluster. These predicted contamination probabilities were 365 above this average percentage, in particular, at 53%. The poor performance is indicative of either 366 inadequate decontamination processes or contamination from the processing environment, 367 equipment or human handling. Lack of sanitizer efficiency in removing or killing pathogens on 368

369 raw fruits and vegetables has also been attributed to structures and tissues that may harbor 370 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 371 hazards to acceptable levels and the overall performance of the FSMS (Luning and Marcelis, 372 2007). Such intervention measures include hygiene and sanitation, and decontamination 373 374 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 375 (40-80 ppm of chlorine) for an average of 5 minutes. However, pH and concentration of chlorine 376 377 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 378 and tolerances with respect to product and process monitoring (Sawe et al., 2014). For chlorine 379 380 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 381 product when active components and conditions necessary for their inactivation are not 382 383 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). 384 385 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 386 (Frank, 2001). In addition, water flumes used during processing can spread initial spot 387 388 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 389 or increase during packing (Johnston et al., 2005). This might therefore explain the presence of 390

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

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

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

436 Water quality was assessed in CSL 5 (incoming water) and 6 (final rinse water), each with a total 437 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 8 438 439 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 440 of positive samples for incoming water was 67% in two factories, and 100% in one factory. 441 Based on statistical modeling results, where each CSL in each processor was again considered as 442 a cluster, the probabilities of contamination in an average cluster, with respect to coliforms and 443 444 *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 445 most clusters the probabilities for coliform contamination were around the estimated probability 446 447 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 448 probabilities in most of the clusters were close to or below the probability in an average cluster 449 450 (33.60%), with the predicted contamination probabilities in two clusters involving incoming 451 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 also had a poor performance at the CSL due to the presence of coliforms and E. coli in the 460 incoming water. Processor P12 had a MSLP of 7 for the CSL while P8 attained a MSLP of 8. 461 Enterococci was detected in the incoming water on all samples drawn from P13, hence a MSLP 462 463 of 6. This might be due to contamination or the presence of biofilms in the piping system (Hallam et al., 2001). Processors P3 and P13 sourced their water from boreholes while the rest 464 used municipal water. There was a better output at CSL 6 (Fig 1f) because all the firms treated 465 466 their water with chlorine prior to using it to rinse the product. In all clusters involving final rinse water, the predicted contamination probabilities were extremely small (only 0.045%). Two 467 processors attained the maximum MSLP of 9 at CSL 6. However, Enterococci were still detected 468 469 in the final rinse water from three processors. The predicted contamination probability in one cluster reached 63% (Fig 4). This may either be due to poor cleaning of flume tanks or 470 ineffective water treatment. 471

472 There was variation in contamination with E. coli. This may be attributed to inadequate cleaning and sanitation as well as cross contamination. Enterobacteriaceae counts at CSL 3 ranged from 473 poor to unsatisfactory (food safety level of 0 or 1) in 80 % of the firms. However, processor P13 474 475 had a good food safety compared to the other companies. Its final product met the criteria throughout the sampling period and cleaning and sanitation was effective with either absence or 476 477 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

481 specifications. Processor P3 had the least performing FSMS as depicted by the MAS results (Fig. 482 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 483 acceptable levels. This poor output was mainly contributed by CSL 3 (food contact surfaces) and 484 CSL 5 (incoming water). Equipment and facilities hygiene is therefore crucial in prevention of 485 486 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 487 contamination to the end product (ICMSF, 2011; Johnston et al., 2005). Hygienic design of 488 489 equipment and facilities must be supported by adequate cleaning and sanitation programmes with performance tests done on a regular basis (Luning et al., 2008). 490 Verification of cleaning and sanitation should therefore be improved for all the processors. 491 Effectiveness of cleaning requires re-validation in order to improve general hygiene and reduce 492 493 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 494 495 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 496 addition, the frequency of cleaning and sanitation should be based on results of verification 497 activities. 498

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

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. A possibility in terms of the survey sampling design would be to consider the factories as clusters, and the critical sampling locations as strata; the above-mentioned calculations could then be conducted. Evidently, the "intra-factory" correlation would be an important input. This correlation could be estimated in the framework of a beta-binomial model (Aerts et al., 2002; Faes et al., 2009; Fitzmaurice et al., 2009; Molenberghs and Verbeke, 2005).

510 4 Conclusion

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

525 direction is suggested where the MAS protocol is utilized in defining the critical sampling

526 locations, with survey sampling principles being used to specify a survey sampling design, and to

527 calculate the number of factories and the number of samples, at each critical sampling location in

528 each factory, that would be needed to achieve various statistical analysis objectives.

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659 Figure Legends

- 660 Figure 1. Microbial safety level profiles at critical sampling locations for fresh produce processing
- 661 firms (a) CSL 1, initial products; (b) CSL 2, final products; (c) CSL 3, product contact surfaces; (d)
- 662 CSL 4, personnel hands and/or gloves; (e) CSL 5, incoming water; (f) CSL 6, final rinse water. P3, P8,
- 663 P9, P12, P13- processors 3, 8, 9, 12 and 13.
- 664 Figure 2 Predicted *E. coli* contamination probabilities in processor-by-critical sampling location
- 665 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

- 667 Probabilities computed from estimates of a logistic regression model with random effects.
- 668 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
- 672 performance levels, together, in each processor-by-critical sampling location combination for
- 673 initial materials and final products
- Horizontal reference lines depict the probability in an average cluster calculated by setting therandom effect in the model to 0 (the mean of the random effects distribution).

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

Table 1. Characteristics of fresh produce processors assessed for microbial performance of safetymanagement 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
		BRC,	BRC,		BRC,
QA standard certified	BRC	CBS	CBS	BRC	CBS
Tonnage exported per					
annum	3000	7800	5000	7000	7000

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

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

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	EC 1441/2007
				g	
2	Final product	E. coli	ISO 7521: 2005	m<10 ¹ ,	EC 1441/2007
				$M < 10^{2}$	
		Salmonella spp	ISO 6579:2002	Absent in 25	EC 1441/2007
				g	
		L. monocytogenes	ISO 11290-	Absent in 25	EC 1441/2007
			2:1998/Amendment	g	

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

		E. coli	ISO 7521: 2005	≤0.7 log	
				$CFU/25 \text{ cm}^2$	
				(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	

^aLFMFP- 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

bacteria per test volume considered marginally acceptable (values at or above M are unacceptable).

Food safety level	^a Criteria	
3	$R \le m$ or organism absent in x grams, milliliters or 50 cm ² or 25 cm ²	
2	m < R < M	
1	$R \ge M$	
^a R, results obtained from	n analysis; m, maximum level of bacteria per test volume considered	
acceptable; M, maximum level of bacteria per test volume considered marginally acceptable		

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

697 (values at or above M are unacceptable)

695