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Microbiological performance of Hazard Analysis Critical Control Point (HACCP)-based food safety management systems: A case of Nile perch processing company

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This study aimed at giving insight into microbiological safety output of a Hazard Analysis Critical Control Point (HACCP)-based Food Safety Management System (FSMS) of a Nile perch exporting company by using a combined assessment, FSMS-diagnosis and actual microbiological assessment. The FSMS diagnosis indicated FSMS activities at an average level operating in moderate-risk context level but with good system output. Likewise, microbiological assessment revealed a better system output with respect to pathogens (*Vibrio cholerae*, *Listeria monocytogenes* and *Salmonella* spp.) and faecal hygiene (*Escherichia coli*) as none of these were detected in any critical sampling location throughout the study. Although indicators of general process hygiene (that is, *Enterobacteriaceae* and TVC) exceeded regulatory limits and guidelines in raw materials and food contact materials, *Staphylococcus aureus* on operator's hands were beyond the general microbiological guidelines in the fish industry. Higher contamination levels of general process hygiene and personal hygiene indicators call for improvement on hygienic design, specific production and sanitation procedures, independent validation, process automation, and change in personnel recruitment criteria.

Key words: Fish export, food safety management system, food safety, microbiological performance.

INTRODUCTION

Globally fish production has significantly increased and contributes to more than 15% of animal source protein (FAO, 2012). In 2010, capture fisheries and aquaculture

supplied the world with 148 million tons of fish valued at US\$217.5 billion (FAO, 2012). The world average per capita consumption of fish has also increased to 18.6 kg

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making fish and fishery products among the most traded food commodities globally (FAO, 2012). Developing countries contribute to the bulk of world fish exports (FAO, 2012). Fish industry is among the largest food manufacturing and exporting sectors in Tanzania (Ruteri and Xu, 2009). The total annual production of fish is estimated at 365,023 tons earning the country about US\$ 800,000 (United Republic of Tanzania, 2013). Tanzania exports 45,550 tons of ornamental fish and 41,291 tons of fisheries products, which worth US\$ 159.1 million (United Republic of Tanzania, 2013). Moreover, fish industry provides substantial employment, income, and foreign exchange contributing to the economic development of the nation. It employs more than 4 million Tanzanians and contributes to about 1.4% national GDP (United Republic of Tanzania, 2010).

Fish importing countries like European Union (EU), United States of America (U.S.A) and Japan have set stringent requirements along the fish market chain (Onjong et al., 2014a). Consequently, exporting countries including Tanzania have taken various initiatives at various levels to translate the requirements into their production systems. At the company level, various quality assurance standards (ISO 22000, BRC, and ISO 9001) and guidelines (HACCP, GMP, and GHP) have been translated into their food safety management systems, FSMS (Kussaga et al., 2014; Onjong et al., 2014a). At the sectoral level, sector organisations like Tanzania Industrial Fish Processors Association (TIFPA) exercised the due diligence in fish safety and quality assurance systems to ensure the quality and safety of export products (www.tifpa.org). At the government level, various regulations were promulgated, the competent authority was designated, workers trained, inspection system improved and landing sites (that is, supplied with potable water, toilets, fenced and paved) were built (Kussaga et al., 2014).

However, despite such efforts, fish companies are still experiencing notifications and border rejections of their products (Rapid Alert System for Food and Feed, 2009b; Kussaga et al., 2014; Rapid Alert System for Food and Feed, 2014b). The major reasons behind such notifications and rejections are filthy, microbiological (like *Salmonella* spp. and *Vibrio cholerae*) and chemical contaminations (pesticides and illegal fishing by using chemical poisons/dynamite) (Rapid Alert System for Food and Feed, 2009b, 2014b). A recent study covering all Tanzanian fish exporting companies identified various inadequacies in the design (hygienic design of equipment and facilities, sampling design and measuring plan, sanitation programmes) and operation (procedures and capability of physical packaging equipment) of core FSMS control activities and set-up of core assurance activities like validation and record keeping system (Kussaga et al., 2014). However, typical microbiological assessment to identify the actual microbiological output of the system was not performed. Therefore, this study

aims at getting deeper insight into the typical causes of insufficient microbiological performance of HACCP-based FSMS of Nile perch exporting company in order to propose intervention measures for improvement towards an effective system. This study involved a combined assessment applying two diagnostic tools; the FSMS-Diagnostic Instrument, FSMS-DI (Luning et al., 2008; Luning et al., 2009; Jacxsens et al., 2010; Luning et al., 2011b) and microbiological assessment scheme, MAS (Jacxsens et al., 2009) to provide a deeper insight in the actual microbiological system output and causes of inadequate performance (Luning et al., 2011a).

MATERIALS AND METHODS

Characteristics of the company analysed

The company analysed in this study processed fresh chilled and frozen Nile perch fillets for the export market. At the time of sampling, this company implemented pre-requisite programmes (PRPs), HACCP and ISO 22000; however, it was not ISO 22000 certified. It is a large-scale company with a total of 150 employees with a daily capacity of processing 120 metric tons (however, currently it processes less than 30 metric tons due to limited availability of Nile perch). It has also a big quality assurance department with 10 personnel and a QA manager. Eventually, this company is approved for export to the EU after being audited by the national competent authority (Department of Fisheries, Ministry of Livestock and Fisheries Development) to determine if the hygiene requirements are in compliance with the EU demands (that is, Commission Regulations (EU) 852/2004, EU 853/2004, and EU 2073/2005). This company was selected over other companies because it agreed to conduct both FSMS diagnosis and microbiological sampling as majority of the companies would not allow for actual microbiological to be conducted. The processing line for the frozen Nile perch fillets (Figure 1) was selected for assessment because at the time of sampling it was the only product being processed. It is also, the major processed product in this company accounting for more than 80%.

Diagnosis of food safety management systems performance

The FSMS-DI is a tool that enables systematic analysis and assessment of a company's specific FSMS (Luning et al., 2008, 2009, 2011b). The diagnostic tool involves a set of 58 indicators representing four crucial parts; part 1 describes set of indicators of context factors including product (3 indicators), process (3), organisational (7), and chain environment (4) characteristics that affect performance of FSMS. Context factors are structural elements of a system environment that can affect decision making activities in the FSMS and system output, and cannot (easily) be changed. The FSMS context is narrower than the overall environment of a company (Luning et al., 2015). For each context indicator a grid was designed including three situational descriptions, corresponding with a low (score 1), moderate (score 2), and high-risk situation (score 3) indicating levels of riskiness for decision-making in the FSMS activities (Luning et al., 2011b). The description for low, moderate, and high-risk situations for product and process characteristics pertains to low, potential, and high likelihood of contamination, growth and survival of pathogens. For organisational characteristics, low, moderate, and high-risk situations respectively represent supportive, constrained/restricted, and lack of administrative conditions to support appropriate

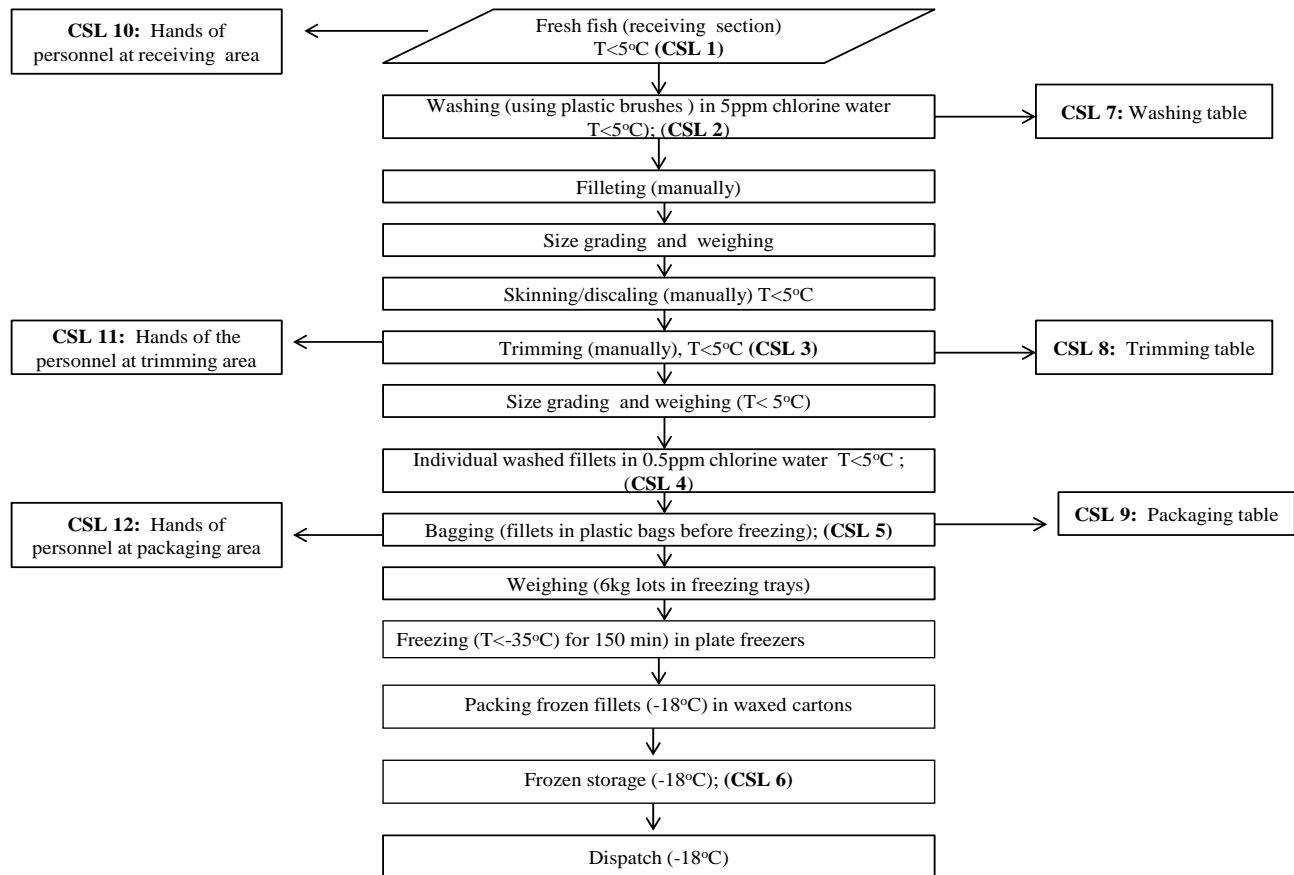


Figure 1. Process flow diagram of frozen Nile perch fillets indicating the critical sampling locations.

decision-making in the FSMS. Concerning chain environment characteristics; low, moderate, and high-risk situations correspond to low, restricted, and high dependability on other chain actors resulting in a more vulnerable decision-making situation, respectively (Luning et al., 2011b).

Part 2 includes sets of indicators that represent core control activities such as design of preventive measures (6), design of intervention processes (4), monitoring system design (8), and actual operation of control strategies (8) (Luning et al., 2008). Control activities are aimed at keeping products and processes within acceptable tolerances. For each control activity indicator a grid with description of four different performance levels, that is, low (score 0), basic (score 1), average (score 2), and advanced (score 3) was constructed (Luning et al., 2008, 2009). A low level represents that an activity is not possible in the given production circumstances (e.g. in freshly packed fish, commonly no physical interventions can be applied), just not applied, or when information is not known. The basic level for control activities is typified by use of own experience, general knowledge, ad-hoc analysis, incomplete, not standardised, unstable, and regularly problems. The average level for control activities is characterised by being based on expert (supplier) knowledge, use of sector/legislative guidelines, best practices, standardised, sometimes problems. The advanced level indicates that the control activity is characterised by use of specific information, scientific knowledge, critical analysis and procedural methods.

Part 3 pertains to set of indicators of core assurance activities including setting system requirements (2), validation (3), verification (2), documentation (1), and record keeping (1) (Luning et al., 2009).

Assurance activities aim at providing evidence and confidence that control activities are effective and function well in actual practice. Likewise, for each assurance activity indicator a grid with description of four different performance levels, that is, low (score 0), basic (score 1), average (score 2), and advanced (score 3) was constructed (Luning et al., 2008, 2009). A low level represents that an activity is not applied, or when information is not known. The basic level is characterised by problem driven, only checking, scarcely reported, and no independent positions. The average level corresponds with active, additional analysis, regular reporting, and experts support. The advanced means that the assurance activity is characterised by use of specific information, scientific knowledge, procedural methods, systematic activities, and independent positions.

Part 4 involves assessment of external (4) and internal (3) system output indicators (Jacxsens et al., 2010). Moreover, for each system output indicator, four levels were described; level 0 (no indication of system output) refers to absent, not present or not conducted. Level 1 (poor system output) is characterised by aspects like ad-hoc sampling, minimal criteria used for FSMS evaluation, and having various food safety problems due to different problems in the FSMS. Level 2 (moderate system output) corresponds to regular sampling, several criteria used for FSMS evaluation, and having restricted food safety problems mainly due to one (restricted) type of problem in the FSMS. Level 3 (good system output) pertains to a systematic evaluation of the FSMS using specific criteria and having no safety problems (Jacxsens et al., 2010). The basic principle behind the FSMS-DI is that companies operating in a high-risk context require core control and

Table 1. Detailed microbial assessment scheme of a frozen Nile Perch fillets processing line.

Critical sampling location	Microbiological parameter	Sampling method
CSL1: Raw fish (in trucks at point of receipt)	Total viable counts (TVC), Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/sampling day) by abrasive swabbing on 50 cm ² of fish skin
CSL2: Raw fish after dipping in 5 ppm chlorine water	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/sampling day) by abrasive swabbing on 50 cm ² of fish skin after disinfection
CSL3: Trimmed fish fillet	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/day) by abrasive swabbing on 50 cm ² of fillet after trimming
CSL4: Trimmed fillet after dipping in 0.5ppm chlorine water	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/day) by swabbing on 50 cm ² of disinfected fillet
CSL5: Bagged fresh fillet before freezing	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/day) by abrasive swabbing on 50 cm ² of bagged fresh fish fillet
CSL6: Final packaged fillet after freezing	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	3 samples (1 sample/day) by abrasive swabbing on 50 cm ² of frozen fillet
CSL7-9: Working tables (receiving, trimming and packaging)	TVC, Enterobacteriaceae, <i>E. coli</i> , <i>V. cholerae</i> , <i>Salmonella</i> spp., and <i>L. monocytogenes</i>	9 samples (3 samples × 3 times/day) by cotton swabs on 25 cm ² of the table, ISO 18593:2004 (ISO, 2004)
CSL 10-12: Hands of operators (receiving, trimming and packaging)	<i>E. coli</i> , Enterobacteriaceae, and <i>S. aureus</i>	9 samples (3 samples × 3 times/day) by cotton swabs on 25 cm ² of personnel hands/gloves, ISO 18593:2004 (ISO, 2004)

assurance activities at an advanced, fit-for-purpose level, whereas in a low-risk context, activities at a lower level could be sufficient to guarantee good system output (Luning et al., 2008, 2009, 2011b).

Microbiological food safety output diagnosis

The principles of the microbial assessment scheme (MAS) protocol developed by Jacxsens and co-authors (Jacxsens et al., 2009) were used to determine the actual microbiological output of an implemented FSMS. Microbiological analysis was conducted at an accredited NFQCL of the Department of Fisheries, Ministry of Livestock and Fisheries Development in Mwanza, Tanzania, which is the competent authority. The next sections clearly indicate the MAS procedure (Table 1).

Selection of critical sampling locations

In this study, 12 critical sampling locations (CSLs) were selected (Figure 1 and Table 1) including the raw materials, the whole fresh fish before offloading from the collection trucks (CSL1), washed whole fresh fish in 5 ppm chlorine water (CSL2), trimmed fresh fillets before washing with 0.5 ppm chlorine water (CSL3), and trimmed fresh fillets dipped in 0.5 ppm chlorine water (CSL4). Other CSLs were bagged fresh fillets before plate freezing (CSL 5) and final packaged plate-frozen fillets (CSL6), tables at receiving (CSL7), tables at trimming (CSL8), and tables at packaging (CSL9), operators' hands at receiving (CSL10) and trimming (CSL11), and operator's hand gloves at packaging (CSL12) areas. Sterile dry enviro-sponges (abrasive) made in USA, 3M St. Paul were used to sample 50 cm² on the products, whereas cotton swabs were used to sample 25 cm² of food contact materials (filtration tray and

surface of filling machine) and hands of the personnel.

Selection of microbiological parameters

Seven microbiological parameters including indicators of food safety (*L. monocytogenes*, *V. cholerae* and *Salmonella* spp.), faecal hygiene (*E. coli*), personal hygiene (*S. aureus*), and general process hygiene (*Enterobacteriaceae* and TVC) were selected.

Sampling frequency

Samples were taken three times in three consecutive months (October 2010 to February 2011). Products were sampled once per sampling day, whereas food contact surfaces and hands of the personnel were sampled three times, that is, start, middle and end of production day (Table 1). A total of 214 samples [(4 samples × 6 (CSL 1-6) × 3 (1 sampling/month in 3 months) + 4 samples × 3 (CSL 7-9) × 3 times of sampling/day × 3 (1 sampling/month in 3 months)) + (1 sample × 3 (CSL 10-12) × 3 times of sampling/day × 3 (1 sampling/month in 3 months))] were taken over the three months period.

Selection of sampling and analytical methods

Sampling and laboratory analysis were conducted according to classical ISO and U.S. Food and Drug Administration-Bacteriological Analytical Manual (FDA-BAM) methods. In this study, non-destructive sampling technique was used for products, food contact surfaces and hands of the personnel. On each product, a sterile template was used to delineate 50 cm² and sterile

pre-moistened dry-sponge (3 M, St. Paul, Minnesota, USA) in the respective dilution medium (as each parameter uses a specific medium) was used to sample vertically, horizontally, and diagonally in the delineated area. Swabbing using abrasive sponges is regarded as the best alternative to destructive/excision sampling (Pearce and Bolton, 2005; Lindblad, 2007). The muscle of a healthy fish is considered sterile (Apun et al., 1999); as the micro-organisms on the surface of fish fillets are a result of cross contamination from personnel, processing water and equipment, and/ or food contact surfaces. Thus, swabbing on the surface of fish fillets by abrasive sponges would give an indication of the level of process hygiene and preventive measures of the company. In low contaminated products, abrasive sponge is superior (in recovering micro-organisms) to dry/wet swab and excision, and it is recommended when contamination levels are not known (Tenhagen et al., 2011). For the food contact surfaces and hands/gloves of the personnel, ISO 18593:2004 (horizontal methods for sampling techniques using cotton swabs on surfaces in food industry) was applied (ISO, 2004). Similarly, a sterile template was used to delineate 25 cm² on working tables whereas pre-moistened cotton swab with respective medium for the specific microbiological parameter was used to sample the delineated area. After sampling, enviro-sponges and cotton swabs were put back into their respective stomacher bags and tubes containing the media. Samples were stored and transported (at ≤4 °C) in a cool box containing ice packs to the laboratory for microbial analysis. At the laboratory ISO 6887-3:2003 standard was used to prepare analytical samples. For detection (absence/presence) tests, 100 mL samples (abrasive sponges for the products) and 5 mL samples (cotton swab for food contact surfaces) were used for laboratory analysis. Enumeration of TVC, *Enterobacteriaceae*, *E. coli*, *S. aureus* and *L. monocytogenes* were respectively carried out by ISO 4833:2003, ISO 21528-2:2004, ISO 16649-2:2001, ISO 6888-1:1999/Amd.1:2003 and ISO 11290-2:1998 standards. Detection of *Salmonella* spp., *L. monocytogenes*, and *V. cholerae* performed according to ISO 6579:2002, ISO 11290-1:1996/Amd.1:2004 and BAM: 1995 standards, respectively.

Data analysis and interpretation

The actual microbiological assessment and FSMS-diagnosis data were analysed by using Microsoft Office Excel 2007. Microbiological results were interpreted according to the criteria described in European Union, Tanzanian and East African Community standards and the guidelines developed by Ghent University (Table 3). With regards to FSMS diagnosis data, the mean scores were calculated and transformed to assigned scores as indicated by Jacxsens et al. (2010) and Luning et al. (2011a). For the indicators of context factors if the mean risk-level is between 1 and 1.2, then score 1 is assigned. If the mean risk-level score is between 1.3 and 1.7, then score 1 to 2 is assigned. If the mean risk-level is between 1.8 and 2.2, then score 2 is assigned. If the mean risk-level is between 2.3 and 2.7, then score 2 to 3 is assigned. Lastly, if the mean risk-level is between 2.8 and 3.0, then score 3 is assigned (Luning et al., 2011a). For the indicators of core FSMS activities and system output, if the mean level is between 0 and 1.2, then an assigned score of 1 is defined. If the mean level is between 1.3 and 1.7, then an assigned score of 1 to 2 is attributed. If the mean level is between 1.8 and 2.2, then an assigned score of 2 is defined. If the mean level is between 2.3 and 2.7, then an assigned score of 2 to 3 is given. Finally, if the mean level is between 2.8 and 3.0, then an assigned score of 3 is attributed (Jacxsens et al., 2010; Luning et al., 2011a). Analysed companies with similar score for each indicator were counted (frequency counting) to get insight into the similarities in the level of design and operation of core FSMS (control and assurance) activities and risk-level of the context wherein the systems operate. The spider web diagrams were

developed by using Microsoft Office Excel 2007 to indicate the risk level of the indicators of context factors and performance levels of the FSMS activities and system output. The medians were also calculated by using Microsoft excel. For comparison purposes, the means and medians of all fish companies (adopted from (Kussaga et al., 2014) are also indicated in Table 3.

RESULTS AND DISCUSSION

Diagnosis of food safety management systems

Figures 3 to 4 illustrate the results of FSMS diagnosis. More coloured spider webs indicate that the indicators of FSMS activities and system output are elaborated at high level or there is high-risk level of the context. This study revealed an average FSMS (median 3, mean 2.2) which operates in a medium-risk context (median 2, mean 1.9) with a subsequent better system output (median 3, mean 3). Likewise, a recent study covering all fish processing companies in Tanzania revealed an average FSMS (median 2.5, mean 2.2) operating in moderate-risk context (median 2, mean 1.9) but with relatively good system output, median 3, mean 2.7 (Kussaga et al., 2014). Although, the FSMS-diagnosis results indicated a better system output, the actual microbial assessment (score 2-3) revealed some inadequacies in the system with regards to indicators of general process hygiene (*Enterobacteriaceae* and TVC), personal hygiene (*S. aureus*) (Figures 4 to 5). However, the current FSMS is effective to pathogens including *L. monocytogenes*, *V. cholerae* and *Salmonella* spp., as none of the pathogens was detected throughout the study. Thus, with regards to pathogens, the current FSMS does not require any further improvement (Jacxsens et al., 2009).

Diagnosis of the risk level of context characteristics

In overall, the FSMS operates in a moderate-risk context (score 2). For product and process characteristics, the company dealt with high-risk raw materials (such as fresh raw fish) and final product groups (like fresh chilled/frozen fillets) which both require special storage conditions to prevent proliferation of micro-organisms including pathogens (median 3, mean 2.7, Table 3 and Figure 2A). Likewise, the national-wide study revealed medium-to-high risk (median 2.7, mean 2.4) product and process characteristics (Table 3). Both raw materials and final product groups are perishables (Jensen et al., 2010). Like other types of fish, Nile perch fish and fresh fillets have high water activity (0.98) and neutral pH, making them good media for microbiological growth (Erkan and Özden, 2008). Moreover, the production process is characterised by small batches with clear interference with people (due to low level of automation in filleting, skinning, and cleaning and disinfection). Besides, the production process has no intervention steps to reduce pathogens to acceptable levels. Under this

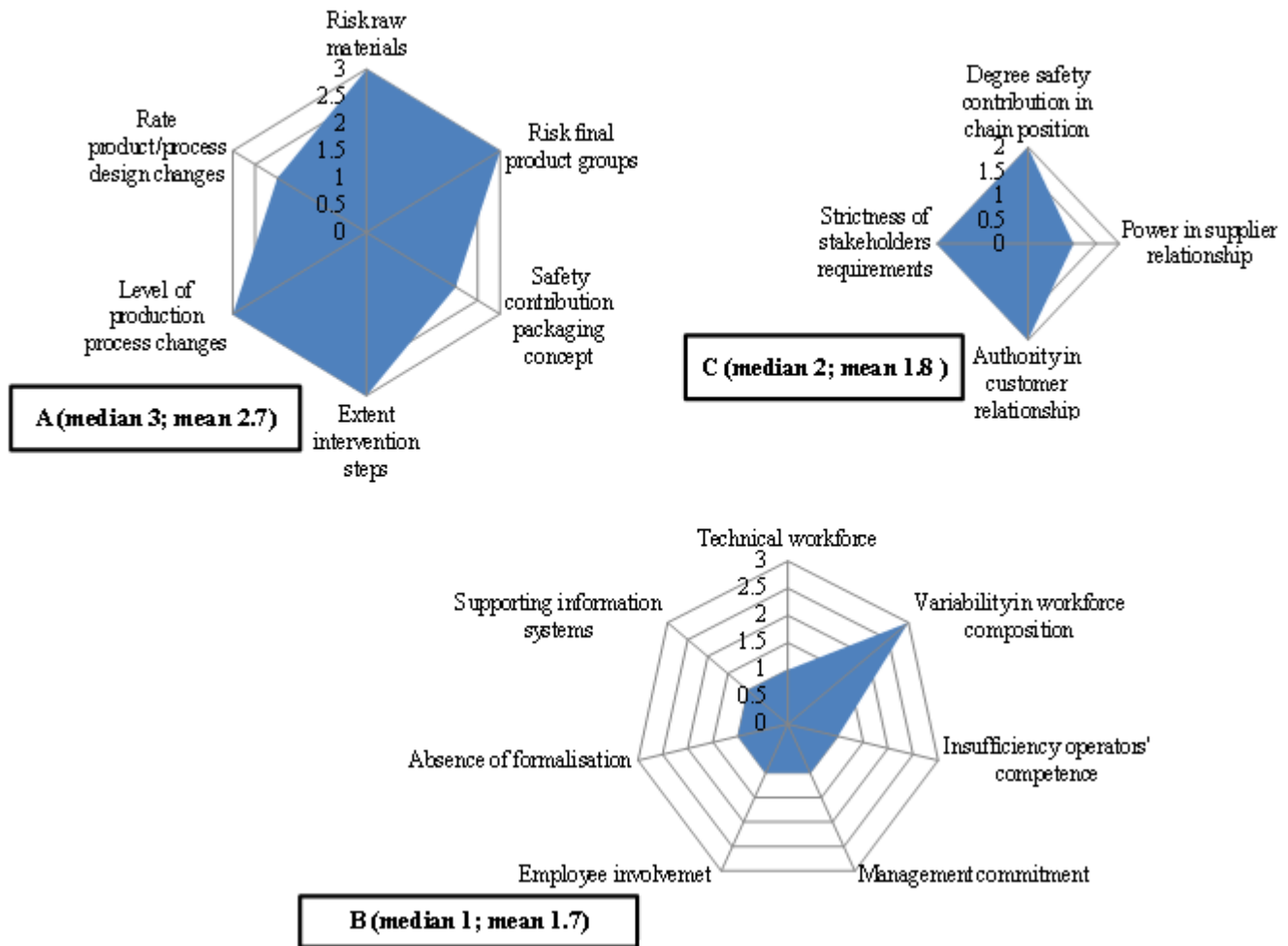


Figure 2. Levels of context riskiness (A) product and process characteristics, (B) organizational characteristics, (C) chain environment characteristics (numbers in brackets indicate median and mean scores).

context situation, it shows that this company is highly dependent on suppliers to ensure quality and safety of its products. Although the company is actively developing supplier specifications, it should also ensure that the preventive control strategies in the FSMS are at an advanced level.

With regards to organisational characteristics, all indicators scored 1 (low-risk level) except degree of variability in workforce composition, which scored 3 (high-risk level; Figure 2B). Like the national-wide study, this study also indicated low-to-medium (median 1, supportive-to-restricted) administrative conditions (Table 3). These administrative conditions support appropriate decision-making in the FSMS due to availability of competent technical staff (trained and experienced), management commitment (food safety/quality policy, food safety team, and financial support), high formalisation (procedures for every activity or operation)

and availability of supporting information systems. However, high-turnover of employees and temporary operators throughout the year increase the chances of poor execution of food safety tasks due to continuous loss of company specific experience/skills. Recent studies observed that majority of fish companies in Tanzania (8/14) (Kussaga et al., 2014) and Kenya (7/9) (Onjong et al., 2014a) had moderate turnover of employees. High variability in workforce composition is also reported in a Vietnamese Pangasius processing company (Thi et al., 2014). As an intervention strategy, the company has to recruit permanent staff and review its remuneration packages and working conditions to enable workers to stay longer. Remuneration packages (like salaries/wages) and working conditions could either motivate workers to perform well and stay longer or frustrate them to quit the job (Mullins, 2007).

For the chain environment characteristics, as observed

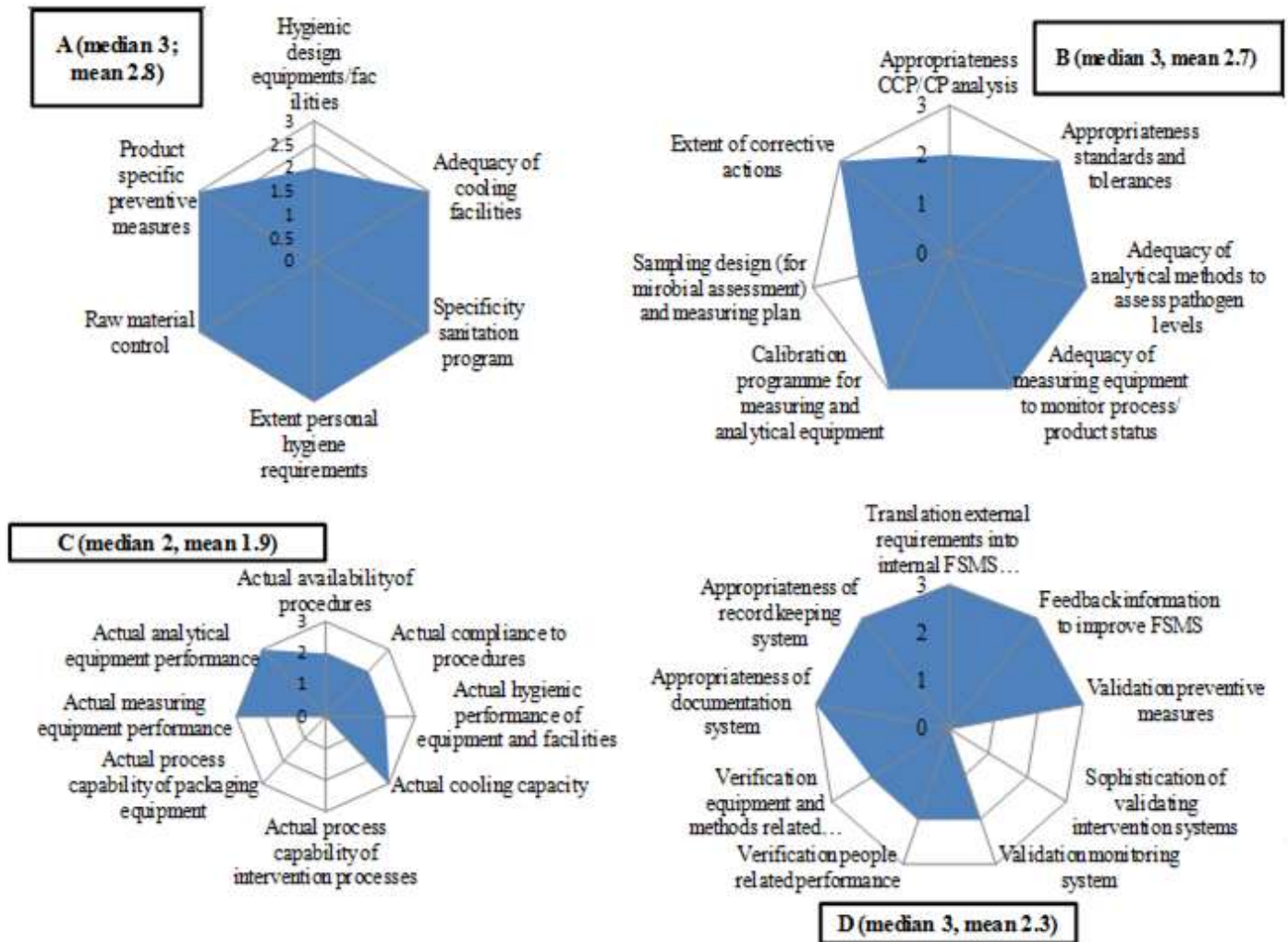


Figure 3. Levels of FSMS activities: (A) preventive measures; (B) monitoring system design; (C) operation of core safety control strategies; (D) assurance activities (numbers in brackets indicate median and mean scores).

in the nation-wide study, the company analysed in this study produced fresh chilled or frozen fillets which require further cooking at the final consumer; thus, it contributes to the final safety through prevention of contamination and growth of pathogens (score 2). With regards to supplier and customer relationships, the company is explicitly involved in the development of product specifications and audit suppliers QMS (score 1). However, it has restricted authority in customers' relationships (as it could only discuss the product use by major customers but has no influence on their systems), and has to meet additional but similar QA requirements from stakeholders like eco-labelling, BRC, HACCP, and traceability (score 2; Figure 2C). Lack of influence on QMS/FSMS of major customers could result into unpredictable use and handling of the products (e.g., temperature abuse, unhygienic handling) compromising safety of the products.

Diagnosis of performance levels of core control activities

All indicators of preventive measures design scored 3 (advanced level) with exception to hygienic design of equipment and facilities, which scored 2, the average level (Figure 3A). In general, this study indicated advanced design of preventive measures (median 3, mean 2.8) as revealed in the nation-wide study (median 3, mean 2.7; Table 3) (Kussaga, 2015). This illustrates that critical equipment like cooling facilities comply with specific hygiene requirements (but not tested in the company specific production situation). Cooling facilities are very critical for food processing companies that do not apply intervention strategies (like heating, fermentation and drying); therefore, their performance need to be tested (Luning et al., 2008). Although offsite assessment revealed that other preventive measures

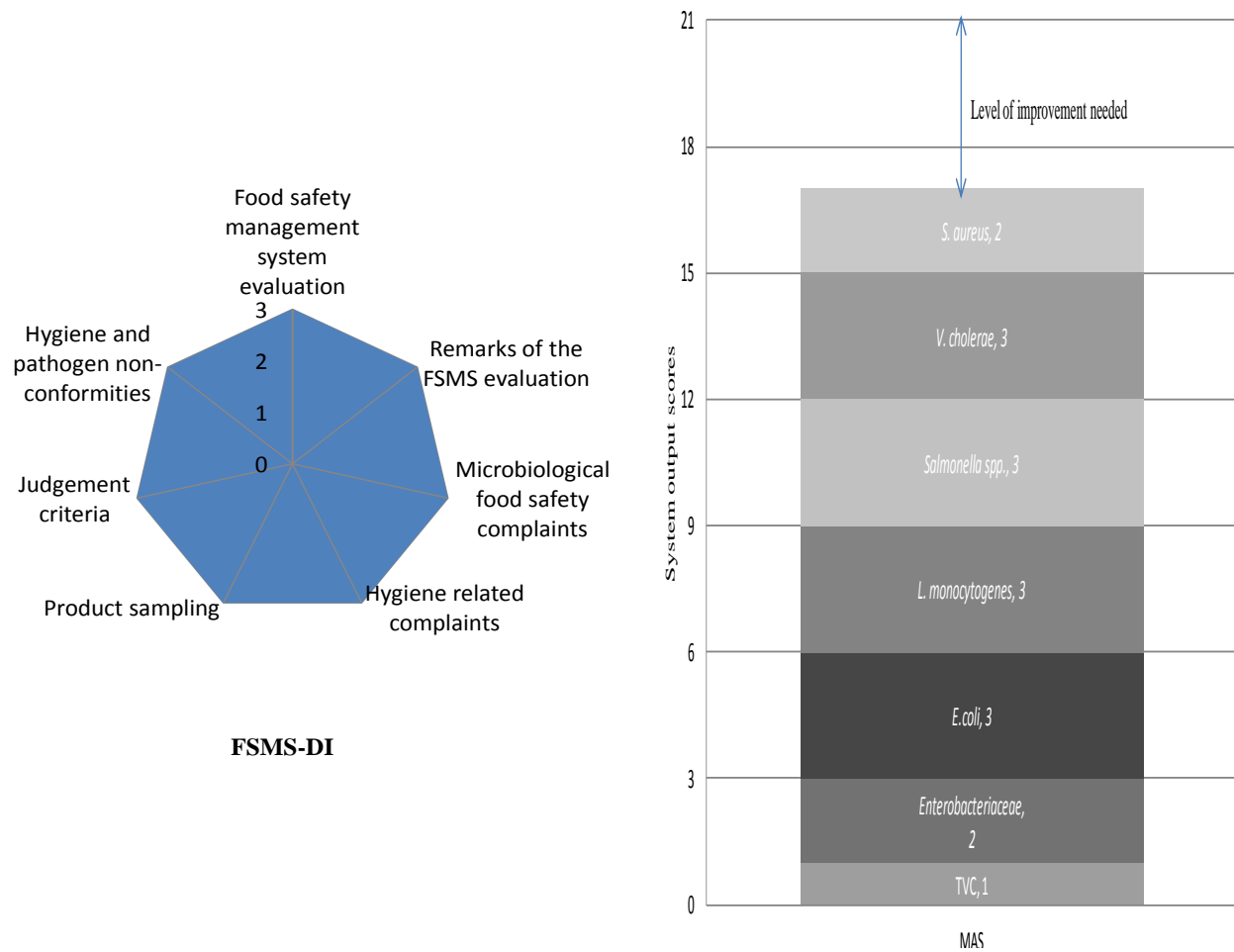


Figure 4. Levels of system output by FSMS-DI (the spider web diagram) and actual microbiological assessment (associated numbers are the scores for each parameter).

were at advanced level, onsite visit showed inadequate cleaning of conveyor belts, flaking out of the wall paints, and condensation from ceiling board, which could serve as potential sources of microbiological proliferation and contamination. In principle, it is required that any equipment in the processing area is included in the cleaning schedule. Moreover, all indicators of intervention processes scored 0 (were not included in calculating the overall FSMS score), because export fish companies in Tanzania process fresh and frozen fish products which do not apply physical intervention processes (like heating) and intervention methods (like fermentation) to reduce microbiological hazards to an acceptable level. Since no intervention processes were applied, the preventive strategies need to be at an advanced level to prevent cross contamination and growth of available micro-organisms (Luning et al., 2011a).

With exception to appropriateness of CCP/CP analysis and specificity of sampling design (for microbiological assessment) and measuring plan (scored level 2), the

rest of the indicators of monitoring system design (corrective actions, standards tolerances, adequacy of analytical methods and measuring equipment, and calibration program) scored level 3 (Figure 3B). Similar situation appears in the nation-wide study which in overall indicated average-to-advanced design of monitoring system (median 3, mean 2.6; Table 3). Analysis of pathogens (like *Salmonella* spp. and *V. cholerae*) and chemical contaminants (pesticides including DDT) is performed by several accredited laboratories including the laboratory of the competent authority, the NFQCL, TBS, and Chemiphar laboratory in Uganda (for heavy metals like lead and mercury). The measuring equipment to monitor process/product status like thermometers were in-line (automated) for the chillers, plate freezers, and cold rooms, where the temperature measurements or variations could be easily seen and temperature records are retrievable. The company has a specific program for calibration and maintenance of thermometers. Normally thermometers are checked on daily basis to ensure

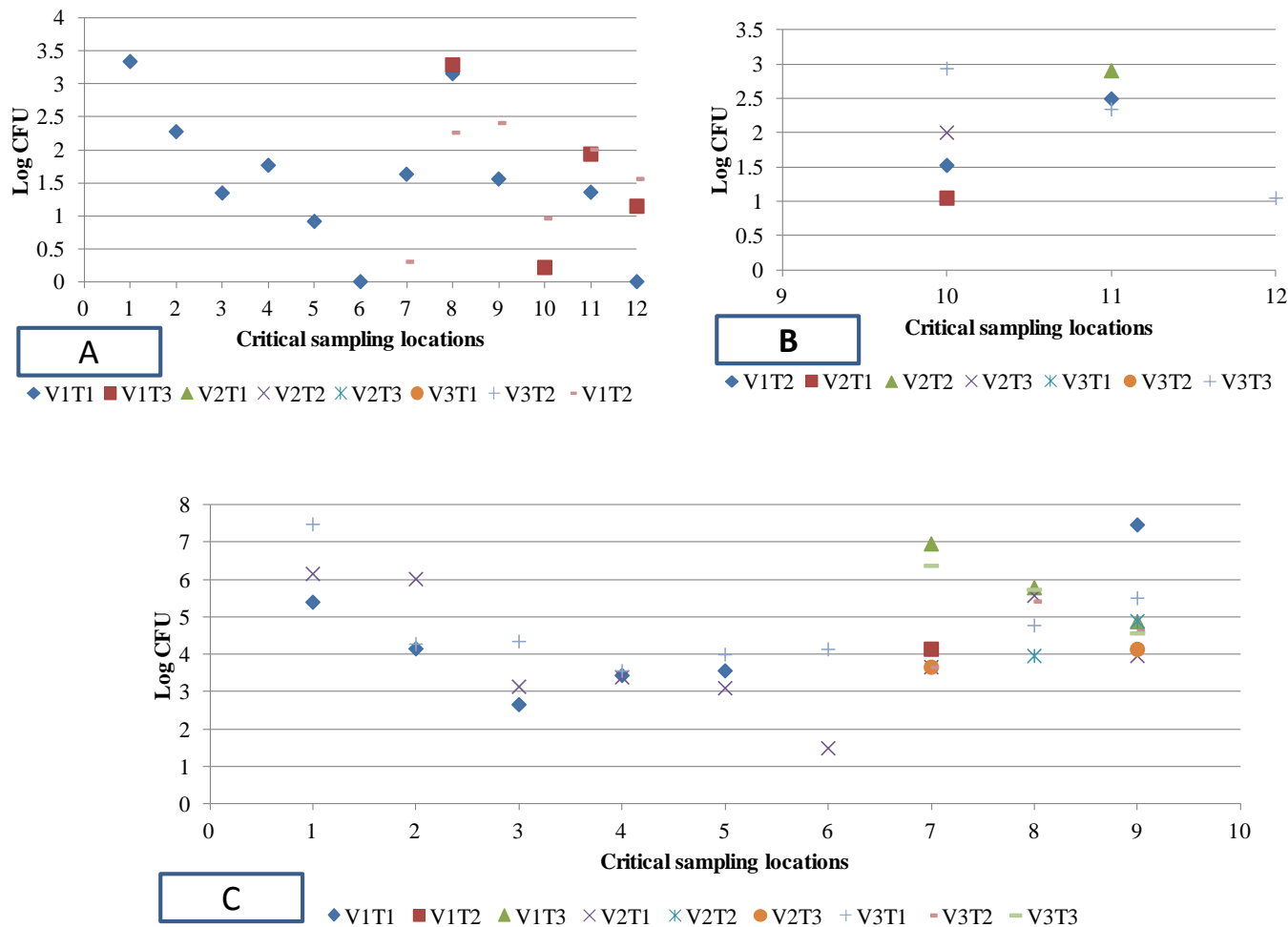


Figure 5. Distribution of (A) *Enterobacteriaceae*, (B) *S. aureus*, and (C) Total viable counts in all critical sampling locations along the frozen Nile Perch processing line. The results are expressed in Log CFU/25 cm² for contact surfaces and Log CFU/50 cm² for products (V1T1- V3T3 (indicate number of visits and times of sampling, e.g. V1T1-visit1 Time1 sampling, V1T2-Visit1 Time2, V1T3-Visit2 Time3, V2T1-Visit2 Time1, V2T2-Viisit2 Time2, V2T3,-Visit2 Time3, V3T1-Visit3 Time1, V3T3,-Visit3 Time2, and 3T3- Visit3 Time3 sampling).

proper freezing of fish products. For audit purposes, calibration and maintenance records are kept up-to-date. In addition, the food control authorities like TBS conduct calibration of measuring equipment (however, periodically). Moreover, the competent authority inspects fish companies on regular basis. A similar study in a *Pangasianodon hypophthalmus* processing company found that sampling design and measuring plan was at an average level (Nosedá et al., 2013). Since analysis of CCP/CPs is done based on expert knowledge without actual testing, the company analysed in this study, could use additional scientific knowledge and experimental tests under the company production circumstances. In addition, the sampling design and measuring plan have to be typified by analysis of pathogen distribution in own food production process.

Like the national-wide study (median 3 and mean 2.2), the company analysed in this study indicated average

design of the operation of control strategies (median 2, mean 1.9 and Table 3). Similarly, the actual process capability of intervention and packaging equipment scored level 0 (Table 3). This is because most fish companies produced fresh and frozen products, so no any physical intervention equipment used. Besides, the packaging concept was not aimed to control or reduce microbial contamination. Fish products were wrapped in plastic bags and packaged in Styrofoam and waxed-box cartons with plastic bag linings to protect them from contaminants (like dirt) and exclude oxygen to prevent oxidation. Moreover, actual availability of procedures, compliance to procedures and hygienic performance of equipment and facilities scored level 2 (Figure 3C). This shows that procedures were available at location though mostly paper-based and kept up-to-date on ad-hoc basis, tasks were executed based on habits and operators regularly controlled on compliance, and unexpected

contamination problems occur due to inappropriate equipment and/or facilities. However, the company had stable cooling capacity and measuring equipment (level 3). The major measuring equipment used were thermometers and pH meters. In addition, the actual performance of analytical equipment scored level 3 because microbiological and chemical analyses were conducted at accredited laboratories of the competent authority for fish products (NFQCL) and national food control agencies (TBS and TFDA). Besides, the company had its own laboratory to conduct basic microbiological analysis (like *Enterobacteriaceae*, *E. coli*, and TVC) with exception to pathogens (*Salmonella* spp. and *V. cholerae*) and chemical contaminants (that is, dioxins and heavy metals like lead and mercury), which are analysed either within (NFQCL, TBS, and TFDA) or outside the country like Chemiphar (U) Ltd in Uganda (especially for heavy metals). Apart from monitoring of chlorine level in processing water (that is, the company has its own water treatment section), other chemical tests (heavy metals) are conducted for monitoring purposes as requested by the competent authority.

Diagnosis of performance levels of core assurance activities

In overall, the nation-wide study (median 2, mean 2.2) indicated similar situation as this study (median 3, mean 2.3 and Table 3). Five out of nine indicators of core assurance activities scored level 3 (Figure 3D). The company scored 0 in validation of intervention systems because no intervention processes were applied. Moreover, it scored level 2 in validation of monitoring system and verification of people- and equipment and methods-related performance. This shows that, however, validation of monitoring system was conducted on regular basis by external expert; it was based on comparison with regulatory documents without experimental trials. Likewise, it confirms that verification activities were conducted on regular basis by independent internal staff by analysing procedures, records and calibration activities. Therefore, this company could develop interventions towards advanced activity levels like scientific based and independent validation of monitoring systems and verification of people- and equipment and methods- related performance. However, as found in the national-wide study (Kussaga et al., 2014), the company proactively translated the external assurance requirements like new legislation (e.g., the EU) and evaluated on its own the critical production circumstances.

Diagnosis of system output by the FSMS-DI

All system output indicators scored level 3 (Figure 4)

indicating good system output (median 3, mean 3 and Table 3). Similarly, a national-wide study revealed a relatively good system output with most indicators scoring level 3 (Table 3) (Kussaga et al., 2014). Based on the self-assessment, this fish company has comprehensive internal and external FSMS output assessment. The FSMS is audited by several accredited third parties including private and governmental (national food control agencies and the competent authority) audits, no major and/or minor remarks on the FSMS, and no customers' microbiological food safety and hygiene related complaints. Besides, the company had structured sampling for both the products and environment, and used combination of legal requirements/criteria and specifications by external parties and company established specifications to judge the microbiological results. Moreover, it had no non-conformities regarding microbiological food safety or hygiene indicators. Fish companies in Tanzania are inspected by the national food safety control authorities and audited by accredited third parties; the majority had specific sampling plans and none experienced microbiological food safety or hygiene non-conformities (Kussaga et al., 2014). The actual microbiological assessment of products, food contact surfaces, and hands/gloves of the personnel were performed to confirm the results of FSMS-diagnosis.

Diagnosis of actual microbiological output of the system

The actual microbiological assessment indicated a moderate-good (score 2 to 3) system output (Table 4 and Figures 4 and 5), which is relatively lower than the one obtained through the FSMS diagnosis (median 3, mean 3; Table 3 and Figure 4). Similar to actual microbiological assessment, a Tanzanian fish nation-wide study indicated an overall moderate-good system output (median 2.5, mean 2.2; Table 3) (Kussaga et al., 2014). This illustrates that although the FSMS-diagnosis revealed advanced activity levels, they are not sufficient to control certain microbiological parameters or deal with the current context risk-level. On the other hand, it reveals an overestimation of the level of design and operation of core control and assurance activities by the company during the self-assessment as it is opposed to the actual microbiological assessment. However, indicators of food safety (*Salmonella* spp., *L. monocytogenes*, and *V. cholerae*) and faecal hygiene (*E. coli*) were respectively below the detection levels (absence in 50 cm² for food products or 25 cm² for food contact surfaces) and quantification limit (<1 CFU in 50/25 cm²) throughout the study (assigned score 3; Table 4). This indicates that the implemented FSMS activities are sufficient to control such microbiological parameters. This is also in agreement with the FSMS-diagnosis, which indicated an average FSMS (median 2.5, mean

2.2) operating under moderate-risk context (median 2, mean 1.9; Table 3). Taking into account that no intervention processes applied, the preventive measures which were the most important control strategies for this company were also at an advanced level (median 3, mean 2.8; Table 3 and Figure 3A).

On the contrary, Enterobacteriaceae were assigned overall score 2 (moderate system output) because were found on tables at trimming (3 out of 9 samples) and packaging (2/9) areas, and hands of personnel at trimming (3/9) and packaging (5/9) sections above the levels in the products handled at the respective areas (Table 4 and Figure 5). The FSMS-diagnosis has also shown restricted use of procedures (which were commonly paper based and not systematically kept up-to-date) as tasks execution was based on habits, and unexpected contamination occasionally occurs due to inappropriate equipment and facilities like flaking out of wall-paints (Figure 3C). Recent studies in fish processing companies in Vietnam (Thi et al., 2014) and Kenya (Onjong et al., 2014b) observed high variability of Enterobacteriaceae on food contact surfaces and hands of the personnel as well as fish products. According to literature, possible causes of Enterobacteriaceae contamination are inadequate procedures of slaughter, handling, packaging, and storage (Boari et al., 2008; Okonko et al., 2008, 2009) and ineffective cleaning of food contact surfaces like tables and equipment (Bagge-Ravn et al., 2003). Likewise, water and ice (Okonko et al., 2009; Shikongo-Nambabi et al., 2010; Mohamed et al., 2011), personnel (Mohamed et al., 2011), and reduced chlorine concentration of the dip after intensive use (Shikongo-Nambabi et al., 2011) or microbial build-up after an extensive use of the dip could be possible causes of contamination. *Staphylococcus aureus* scored level 2 because were observed on hands of personnel at receiving (4/9 samples), trimming (2/9) and packaging (1/9, Table 4; Figure 5) above the microbiological guidelines in the fish industry (Table 2), indicating inadequate personal hygiene. Also, FSMS diagnosis revealed high turnover of employees and execution of tasks were based on habits, indicating that good manufacturing and hygienic practices (like personal hygiene, hand washing, use of aprons/hair covers) were not exactly followed. Previous studies reported *S. aureus* on workers hands and fishery products (Simon and Sanjeev, 2007; Mohamed et al., 2011; Onjong et al., 2014b; Thi et al., 2014). Food handlers are also known to be potential sources of staphylococcal food contamination (Okonko et al., 2009; Adedeji and Ibrahim, 2011; Mohamed et al., 2011).

Total viable counts exceeded the limits in raw materials (1/3 samples) and tables at trimming (7/9) and packaging (7/9) sections. The huge variations in TVC was noted in products (2.4 to 7.5 log CFU/cm²) and working tables (<1-7.5 log CFU/cm²) (assigned score 1, Table 4 and Figure 5). However, high prevalence of TVC on working

tables suggests that the company has inadequate pre-requisite programs (PRPs) particularly, the raw material purchasing specification and cleaning and disinfection. Although water could be another route of contamination, the possibility of contamination through processing water is minimal as the company routinely monitors microbiological quality and chlorine level in the water. Besides, the company has its own source of water and treatment is done and monitored by the company. Though the company dealt with high-risk raw materials (which could be contaminated from the source and along the chain, and require special storage conditions; Figure 2A), there were no intervention processes applied (as the 5 ppm concentration of chlorine could not reduce microbiological levels to an acceptable level). This chlorine concentration (5 mg/L) is also far below the EU levels of chlorine (250 mg/L in form of chloride) required in drinking water (European Union Commission, 1998). Furthermore, the company had restricted hygienic design of equipment and facilities and no independent verification of equipment/methods and people related performance. According to literature, skin or fillet of freshly caught fish may contain microbial load ranging from 2 to 6 log CFU/cm² (Olafsdóttir et al., 1997). The bacterial loading on freshly caught fish reflects the environment from which it was caught, rather than the fish species (Al-Harbi and Uddin, 2005). Also, other studies noticed TVC beyond the set standards in raw fish (Okonko et al., 2009; Shikongo-Nambabi et al., 2010; Onjong et al., 2014b), fresh fish-fillets (Chytiri et al., 2004; Onjong et al., 2014b), working tables (Okonko et al., 2009; Onjong et al., 2014b) and hands of the personnel (Okonko et al., 2009). Thus, raw materials, food contact surfaces, and hands of personnel could be the sources of TVC (Chytiri et al., 2004; Shikongo-Nambabi et al., 2010; Shikongo-Nambabi et al., 2011). Furthermore, this company (including other Tanzanian fish exporting companies) occasionally receives notifications and border rejections of their products due to failures to meet microbiological standards of the export market (Food and Veterinary Office, 2007; Kadigi et al., 2007; Rapid Alert System for Food and Feed, 2009a; Day et al., 2012; Kussaga et al., 2014; Rapid Alert System for Food and Feed, 2014a). In general, improving the PRPs would address the food safety problems reported in this study.

Conclusions

Although the design and operation of FSMS activities sufficiently controlled some microbiological parameters, the actual microbiological assessment indicated slightly low system output as compared to the FSMS diagnosis. The actual microbiological assessment found variable and high counts of TVC in raw materials, final products and food contact surfaces as well as Enterobacteriaceae in food contact surfaces.

Table 2. Microbiological specifications of fish products, food contact surfaces, and hands of the personnel.

Microorganisms	Maximum limit (CFU)			
	Tanzanian Standards ^a	East African Standards ^b	USFDA ^d	Ghent University guidelines ^e
Fresh fin fish				
Total viable counts	m = 10 ⁶ CFU/g, M = 10 ⁷ CFU/g	M = 10 ⁶ CFU/g	-	-
<i>E. coli</i>	m = 5 CFU/g, M = 10 ² CFU/g	M = 10 ¹ CFU/g	-	-
<i>Enterobacteriaceae</i>	-	M = 10 ² CFU/g	-	-
<i>L. monocytogenes</i>	-	-	Absent in 25 g	-
<i>Salmonella</i> spp.	Absent/g	Absent in 25g	Absent in 25 g	-
<i>V. cholerae</i>	-	Absent in 1g	Absent in 25 g	-
Frozen fin fish^b or fillets^f				
Total viable counts	m = 10 ⁶ CFU/g, M = 10 ⁷ CFU/g	M = 10 ⁶ CFU/g	-	-
<i>E. coli</i>	m = 5 CFU/g /g, M = 10 ² CFU/g	M = 10 ¹ CFU/g	-	-
<i>Enterobacteriaceae</i>	-	M = 10 ² CFU/g	-	-
<i>L. monocytogenes</i>	-	-	-	-
<i>Salmonella</i> spp.	Absent	Absent in 25 g	-	-
<i>V. cholerae</i>	-	Absent in 1 g	-	-
Food contact surfaces (working tables)				
Total viable counts	-	-	-	**
<i>E. coli</i>	-	-	-	**
<i>Enterobacteriaceae</i>	-	-	-	**
<i>L. monocytogenes</i>	-	-	-	Absent in the tested area
<i>Salmonella</i> spp.	-	-	-	Absent in the tested area
<i>V. cholerae</i>	-	-	-	Absent in the tested area
Hands of the personnel				
<i>E. coli</i>	-	-	-	**
<i>Enterobacteriaceae</i>	-	-	-	**
<i>S. aureus</i>	-	-	-	Below limit (10 CFU/25 cm ²) of quantification

^a(Tanzania Standard, 1988), ^b(East African Community, 2010a), ^c(European Union, 2005), ^d(U.S. Food and Drug Administration, 2009), ^e(Sampers et al., 2010), ^f(East African Community, 2010b); **Same as product handled in the respective area.

Table 3. Median and mean scores of context factors, control and assurance activities, and system output of fish processing companies in Tanzania.

Context factor and core control or assurance activities	Median and Mean (in brackets) of all (14) fish companies*	Median and mean (in brackets) of single company
Context characteristics		
Product and process characteristics	2.7 (2.4)	3.0 (2.7)
Organisation characteristics	1 (1.5)	1 (1.3)
Chain-environment characteristics	2 (1.7)	2 (1.8)
<i>Overall context-riskiness</i>	2 (1.9)	2 (1.9)
Core control and assurance activities		
Preventive measures design	3 (2.6)	3 (2.8)
Intervention measures	0 (0.87)	0 (0.8)
Monitoring system	3 (2.6)	3 (2.7)
Actual operation of core control strategies	3 (2.2)	2 (1.9)
Assurance activities	2 (2.2)	3 (2.3)
<i>Overall FSMS performance</i>	2.5 (2.2)	3 (2.2)
System output	3 (2.7)	3 (3.0)

*Adapted from Kussaga (2015).

Table 4. Detailed MAS results indicating microbial parameters analysed at each CSL, frequency of detection/quantification, and assigned and overall system output scores.

Critical sampling location (CSL)	Detection of food safety indicators			Quantification of indicators of hygiene (CFU/50 or 25 cm ²)**			
	Absent (A)/Present (P) in 50 or 25 cm ²			Faecal hygiene	Personal hygiene	Overall process hygiene	
	LIST ^a	SALM ^b	VIBRIO ^c	ECOL ^d	STAP ^f	ENTE ^e	TVC ^g
1. Raw fish (before washing)	A	A	A	<1	NA	<1-3.3	5.4-7.5 (1/3) ^h
2. Washed raw fish	A	A	A	<1	NA	<1-2.3	4.1-6.0 ⁱ
3. Trimmed fillets	A	A	A	<1	NA	<1-1.3	2.7-4.3
4. Washed fillet	A	A	A	<1	NA	<1-1.7	3.4-3.6
5. Bagged fillet	A	A	A	<1	NA	<1	3.1-4.0
6. Packaged frozen fillet	A	A	A	<1		<1	<1-4.1
7. Tables at receiving section	A	A	A	<1	NA	<1-1.6	<1-6.9
8. Tables at trimming section	A	A	A	<1	NA	<1-3.3 (3/9)	<1-5.8 (7/9)
9. Tables at packaging section	A	A	A	<1	NA	<1-2.4 (2/9)	<1-7.5 (7/9)
10. Operator's hands- receiving section	NA	NA	NA	<1	<2 -2.9(4/9)	<1-1.0	NA
11. Operator's hands- trimming section	NA	NA	NA	<1	< 2-2.9 (2/9)	<1-2.3 (3/9)	NA
12. Operator's gloves- packaging section	NA	NA	NA	<1	<2	< 1-1.6 (5/9)	NA
Total samples not detected with pathogens or microorganisms below or within the legal limits	45/45	45/45	45/45	72/72	20/27	59/72	26/45
Total samples detected with pathogens or microorganisms exceeding the legal limits	0/45	0/45	0/45	0/72	7/27	13/72	19/45
System output assigned score	3	3	3	3	2	2	1
Overall system output score	17/21 (score 2-3)						

^a *L. monocytogenes*; ^b *Salmonella* spp.; ^c *V. Cholerae*; ^d *E. coli*; ^e *Enterobacteriaceae*; ^f *S. aureus*; ^g Total viable counts; ^h number of samples exceeding the limit in all samples analysed within a particular CSL; ⁱ lowest and highest CFU counted in all three visits within a specific CSL; NA - not applicable; ** The results are expressed in log CFU/50 cm² for products and log CFU/25 cm² for contact surfaces (filtration tray, filling machine) and hands of personnel. Bolded numbers indicate samples that exceeded legal limits or guidelines. Tanzania standards were used to interpret results for TVC and *E. coli* in raw fish and frozen fillets; East African standards for *L. monocytogenes*, *Salmonella* spp., *E. coli* and *Enterobacteriaceae* in frozen fish fillets, European Union for *Salmonella* spp., *V. cholerae* and *L. monocytogenes* in raw fish; Ghent University guidelines for *Enterobacteriaceae*, *Salmonella* spp. *V. cholerae*, and *L. monocytogenes* on food contact surfaces and *S. aureus* on hands of the personnel (Table 2).

Currently, there are no EU requirements set for such parameters, providing an opportunity for this Nile perch processing company to continue exporting to the EU as pathogen levels are within the EU standards. However, higher levels of Enterobacteriaceae indicate possibilities of health issues as these are regarded as indicators of process hygiene, inadequate processing and post

processing contamination. If there is poor process hygiene there is a chance of introducing pathogens to the process, or when the heating process was inadequate survival of pathogens is likely. Some members of the family Enterobacteriaceae (e.g. *Shigella* spp.) are also responsible for causing foodborne diseases. The level of context riskiness could be reduced

through automation of the production process (like filleting, packaging, and sanitation) to reduce personnel interferences, recruitment of trained and experienced personnel on permanent basis, and specify product-use by major customers (storage and distribution conditions). The levels of FSMS activities could be enhanced through re-designing of equipment (like automation) and

facilities (re-painting and filling cracks on the walls and floors), improving sanitation programme (including all equipment in the cleaning and sanitation schedule), changing sampling design and measuring plan (by analyzing pathogen distribution in the production process), improve procedures (specifically designed for user, easily accessible, well understood and internalised), independent validation (experimental trials, well established and documented) and verification (supported by scientific evidence and data from own food production process). Therefore, fish companies are required to improve the level of the design and operation of the FSMS activities and reduce the level of context riskiness to guarantee good system output that will ultimately reduce microbiological notifications on fish export.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

FSMS, Food safety management system; **FSMS-DI**, food safety management system - diagnostic instrument; **CSL**, critical sampling location; **TVC**, total viable counts; **TIFPA**, Tanzania Industrial Fish Processors Association; **QA**, quality assurance; **FDA-BAM**, U.S. Food and Drug Administration-Bacteriological Analytical Manual; **MAS**, Microbial Assessment Scheme; **QMS**, Quality Management System; **NFQCL**, National Fish Quality Control Laboratory; **TBS**, Tanzania Bureau of Standards; **TFDA**, Tanzania Food and Drugs Authority.

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