



Effective Operation of Food Quality Management System: A Case Study from Fishery Processing

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Abstract

Performance of food safety management system (FSMS) by Self diagnostic instrument (DI) and Microbial assessment scheme (MAS) are still new approaches in Vietnamese *Pangasius* processing industries. Performance variability of food safety management systems (FSMS) in practice during processing steps makes microbial quality and safety of *Pangasius* products very challenging. Therefore, 117 samples of fish, water and environment were collected throughout processing to assess the effective operation of the FSMS in practice. The dynamics and variations in the microbial quality and safety were observed. The microbial count of the final products ranged 6.8-7.7 log CFU/g of total mesophilic count, <1-<2 log CFU/g of *Escherichia coli*, <1-6.3 log CFU/g of *Coliform* and <2-4.6 log CFU/g of *Staphylococcus aureus*. High prevalence of pathogens was observed on processed fish; 15/36 *Listeria monocytogenes* and 1/36 *Salmonella spp.* with a similar trend in food contact surfaces, hands of operators and water. More attention should be focused on this company because the current FSMS is not performing effectively by means of Self-Diagnostic Instrument (DI) and Microbial Assessment Scheme (MAS) tools. These assessment tools are necessary to implement routinely to validate the FSMS in place.



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Self- Diagnostic Instrument.

Introduction


Pangasius hypophthalmus (or tra fish) is one of the major fish species in the Mekong River fishery and most important inland fisheries in the world.¹

However, despite the success in the production and exportation of *Pangasius* fish in Vietnam, the knowledge of microbiological quality and safety during processing is still very minimal and the safety

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of the exported *Pangasius* fillets remains in question. Reviewed literature shows that only a few studies have been conducted in the microbial safety and quality of *Pangasius* fish and its products and the control of (cross) contamination by pathogens is still challenging.^{2,3} Vietnamese *Pangasius* companies processed *Pangasius* products such as *Pangasius* fillets, slices, portions etc. and export to more than 100 countries world-wide.⁴ Given its economic importance in the country as whole, enough research should be conducted to understand the microbial profile of *Pangasius* fish and their dynamics during processing and therefore reducing the research gap observed in *Pangasius* fish industry.

To accomplish food and nutrition security with European, various diagnostic tools including self-diagnostic instrument (FSMS-DI) and microbiological assessment scheme (MAS) were developed to evaluate performance of Food Safety Management System (FSMS)⁵⁻⁷ and later extended beyond Europe.⁸ However, the performance of FSMS by these tools, still remain new approaches in Vietnam despite the implementation of several FSMSs such as HACCP, BRC, IFS, ISO 9001, ISO 14001.² In the current study, core assurance and control activities, the riskiness of context factors and system output was diagnosed by using FSMS-DI.⁹⁻¹¹ To find out if FSMS were efficiently implemented, the evolution and variation of microbial counts throughout the processing chains using MAS was analysed.⁶ *Pangasius* products faced several setbacks associated with food safety due to rejection when exported to European countries such as shown in the Rapid Alert System for Food and Feed (RASFF). Therefore, Self-diagnostic instrument and microbial assessment scheme were used to evaluate the FSMS of *Pangasius* fish processing company and the results were used for in-process traceability to identify critical locations, process and practices then set in strategies for improvements.

Material and Method

Processing Company

In this study, the *Pangasius* processing plant sampled was located in Vinh Long city, the southern part of Vietnam. The production capacity of the company is 70 to 100 tons per day with approximately 1000 workers in the processing area. Seventy percent (70%) of the company products are exported to

China and the remaining 30% to Italy, Germany and Poland. In addition, the company is HACCP certified.

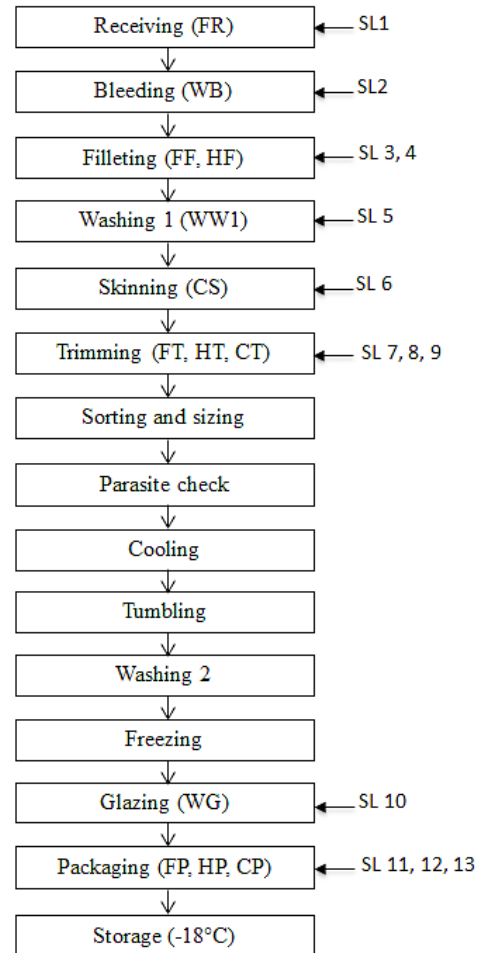


Fig.1: Flow chat for *Pangasius* products and critical sampling location (SL)

The live fish were transported from farms to the processing factory by boat and rarely by trucks. Upon arrival at the company, the processing steps were as shown in the processing chart (Fig.1). After receiving, the fish were bled by manual throat-cutting and thrown in a continuous flow of water then quickly filleted. The fillets were washed in a water bath containing tap water then automatically skinned. Trimming was done manually to remove any remaining skin, subcutaneous fat, red muscles as well as shaping the edges of the fillets. Sorting was done according to size and weight then parasites checked by placing the fillets on a translucent table

illuminated from below. The fillets were then cooled in large industrial ice boxes using flake ice then tumbled in rotating tanks while being treated with unspecified additives for few hours. The treated fillets were then washed in stationary tap water and prepared for freezing in an individual quick freezer (IQF, Mycom, Japan). A core temperature of the fillets was achieved at -18°C . The frozen fillets were then glazed in ice water for one minute before packaging. The frozen products were packed into carton boxes, labeled and stored at -18°C .

Diagnostic Self-Diagnostic Instrument (FSMS-DI)

Level of contextual situation, FSMS activities and Food Safety Performance Indicators (FSPI) were diagnosed by FSMS-DI to obtain the first indication about the microbial performance of FSMS present in the company.^{5,7,9,10} The quality assurance manager was interviewed for four hours. The questionnaire developed by these studies^{5,9} which consists of four assessment parts: *contextual factors*, *core control*, *core assurance activities* and FSPI was used. To illustrate visually the contextual situation, FSMS activities, and FSPI, the results of FSMS-DI as obtained by the assigned score of the given indicators was used to make spider web diagrams.

Microbial Assessment Scheme (MAS)

The principle of MAS is that, low numbers and small variations in microbial counts reveal an efficient system.^{6,10} It gives the actual microbiological performance of an FSMS as an indication of their food safety output. Microbial analysis of the fish fillets, water and environmental samples was conducted using procedures explained by the researchers.⁶

Selection of Critical Sampling Locations

The critical sampling locations (SL) are locations where the loss of control will lead to unacceptable food safety problems due to contamination, growth and/or survival of microorganisms. The raw materials, semi products, final products, food contact materials and contact hands/gloves indicated by SL 1-SL 13 (Fig.1).

Selection of Microbiological Parameters

The total mesophilic count (TMC), *E. coli*, *coliform*, *S. aureus*, *L. monocytogenes*, *Salmonella spp.* and

V. cholerae were investigated in fish and hands/gloves samples collected from different critical sampling locations as shown in Fig. 1 using criteria developed by Laboratory of Food Microbiology and Food Preservation (LFMFP)¹² and official standard established by Vietnamese Science and Technology Ministry 8338.¹³ For water and food contact surfaces, TMC, *E. coli*, *coliform*, *L. monocytogenes*, and *Salmonella spp.* were investigated.

Sampling Frequency

Each processing line was sampled at three different times in three consecutive weeks (8 a.m., 12 p.m., and 2 p.m. - three visits per sampling day). The total of 117 samples was collected, 36 samples being from fish (raw fish and fillets), 27 samples from water, 27 samples from food contact surfaces and 27 samples from hands/gloves of food operators. The experiment was performed in July–September 2019.

Sampling and Analysis Method

For fish samples, a whole *Pangasius* fish (during receiving) and $200\text{g} \pm 20\text{g}$ fillets (*Pangasius* fillets during filleting, trimming and packaging) were taken and placed aseptically in stomacher bag with sterile tweezers for further analysis. Samples for hands and food contact surfaces were collected aseptically from hands/gloves, machines surfaces and utensils used during processing by swab method vertically, horizontally and diagonally each time on 50 cm^2 surface in 5ml Maximum Recovery Diluent (MRD, Merck, Darmstadt, Germany) for analysing TMC, *E. coli*, *coliform* and *S. aureus*; in 5ml Demi-Fraser medium (Merck, Darmstadt, Germany) for detection *L. monocytogenes*; in 5 ml Buffered Peptone Water (BPW, Merck, Darmstadt, Germany) for detection *Salmonella spp.* and 5 ml Alkaline Saline Peptone Water (ASPW, Merck, Darmstadt, Germany) for detection *V. cholerae*. For water samples, 500 ml of water samples were aseptically collected from bleeding, washing and glazing steps. The samples were aseptically stored in ice and transported in insulated boxed to Department of Food Technology, Can Tho University, Vietnam for microbiological analyses within 6 to 24 h of sampling.

Enumeration of Microorganisms by Quantitative Analysis

Fish samples (25g) were taken from different parts of the fish or fillets using sterile scalpels and tweezers.

Swab and water samples were vortexed for about 10s, and tenfold serial dilution for fish, water and swab samples were made in MRD.

The total mesophilic counts were determined using Plate Count Agar (PCA, Merck, Darmstadt, Germany) and incubated at 37°C for 2-3 days. Enumeration of *E. coli*/Coliform was plated on Coliform Agar ES (Enhanced selectivity) (Merck,

Darmstadt, Germany) by incubating for 24h at 37°C. *S. aureus* was enumerated by spread plating on Baird Parker Agar (Merck, Darmstadt, Germany) with 25ml/500ml Egg Yolk Tellurite Emulsion (Merck, Darmstadt, Germany), and after an incubation period of 48h at 37°C and confirmation of *S. aureus* occurred with a Bactident® Coagulase positive (Merck, Darmstadt, Germany).

Table 1. Microbiological criteria or guideline values for microbial interpretation²

| Microbial parameters | Fresh fish in Belgian food industry ^b (log CFU/g) | | Frozen <i>Pangasius</i> fish fillet ^v (log CFU/g) | Food contact surface ^b (log CFU/100cm ²) |
|-------------------------------|--|----------------|--|---|
| | Goal | Tolerance | Tolerance | |
| Total mesophilic counts | 5 | 6 | 6 | Good, ≤ 3; moderate 3-4.5; poor ≥ 4.5 |
| Enterobacteriaceae//Coliform* | 2 | 3 | - | Good, ≤ 3; moderate 3-4.5; poor ≥ 4.5 |
| <i>E. coli</i> | 2 | 3 | 2 | Absence in area tested |
| <i>Staphylococcus aureus</i> | 2 | 3 | 2 | Absence in area tested |
| <i>V. cholerae</i> | - | - | Absence in 25g | Absence in area tested |
| <i>L. monocytogenes</i> | Absence in 25g | - | Absence in 25g | Absence in area tested |
| <i>Salmonella</i> spp. | Absence in 25g | Absence in 25g | Absence in 25g | Absence in area tested |

^bAccording to guideline value for fresh fish developed by the Laboratory of Food Microbiology and Food Preservation (Ghent University)¹²

^vAccording to microbiological criteria for production frozen Tra fish (*Pangasius hypophthalmus*) fillets established by Vietnamese Science & Technology Ministry 8338-TCV¹³

*No guidance value or criteria for Coliforms; thus the guidance value of Enterobacteriaceae can be used for Coliforms

- not mentioned in the guideline for fresh fish or the criteria for frozen *Pangasius* fish

Qualitative Analysis

The microbial analysis of *V. cholerae* followed ISO 21872-1:2017. To determine the strains of *V. cholerae* biochemical confirmation (i.e. Gram-negative, oxidase-positive) was done. For *L. monocytogenes* ISO 11290-2:2017 was followed using Listeria agar (Merck, Darmstadt, Germany) and that of *Salmonella* followed the ISO 6579-1:2017 using Xylose Lysine Deoxycholate agar and sent to an external agent for biochemical confirmation and serotyping using Triple Sugar Iron (TSI), Indole test, Lysine decarboxylase

(LDC), Ortho-nitrophenyl-/3-D-galactopyranoside (ONPG), Urease test, O-antigens and H- antigens.

The results for self-diagnostic instruments were transformed to an assigned score for *contextual factors*, FSMS activities and FSP.^{16,10} The overall indication of FSMS of the sampled company was given by the assigned scores and to search for possible improvement points, individual scores were taken into account.¹¹

The results of the microbial analysis for each selected parameter in each critical sampling location were compared and judged against the criteria/guidelines for fresh fish; hands or food contact surfaces as shown in Table 1. According to EU Council Directive 98/83/EC¹⁴ and Vietnamese regulation,¹⁵ the initial quality of water used for washing or glazing fish must meet potable water standards. The microbial safety level (ranging from level 1 to 3) was assigned based on the method developed by Jacxsens, Kussaga, Luning, Van der Spiegel, Devlieghere, Uyttendaele.⁶ When there is no legal criteria, microbial guidelines established by the LFMFP, Ghent University was used.¹² A microbiological safety level profile

was calculated by summation of assigned to a total of 21 (3 levels × 7 microbial parameters). For microbiological profile lower than 21, improvement of the FSMS will be advised.

Statistical Analysis

The results of the microbial analysis of fish (log CFU/g), water (log CFU/ml), and hands/ food contact surface (log CFU/100 cm²) samples are represented as the mean value ± standard deviation. Differences in mean value throughout the different visits and independent sampling times were statistically assessed using SPSS version 20 (IBM Inc., Chicago, Ill., USA) ($\alpha = 0.05$).

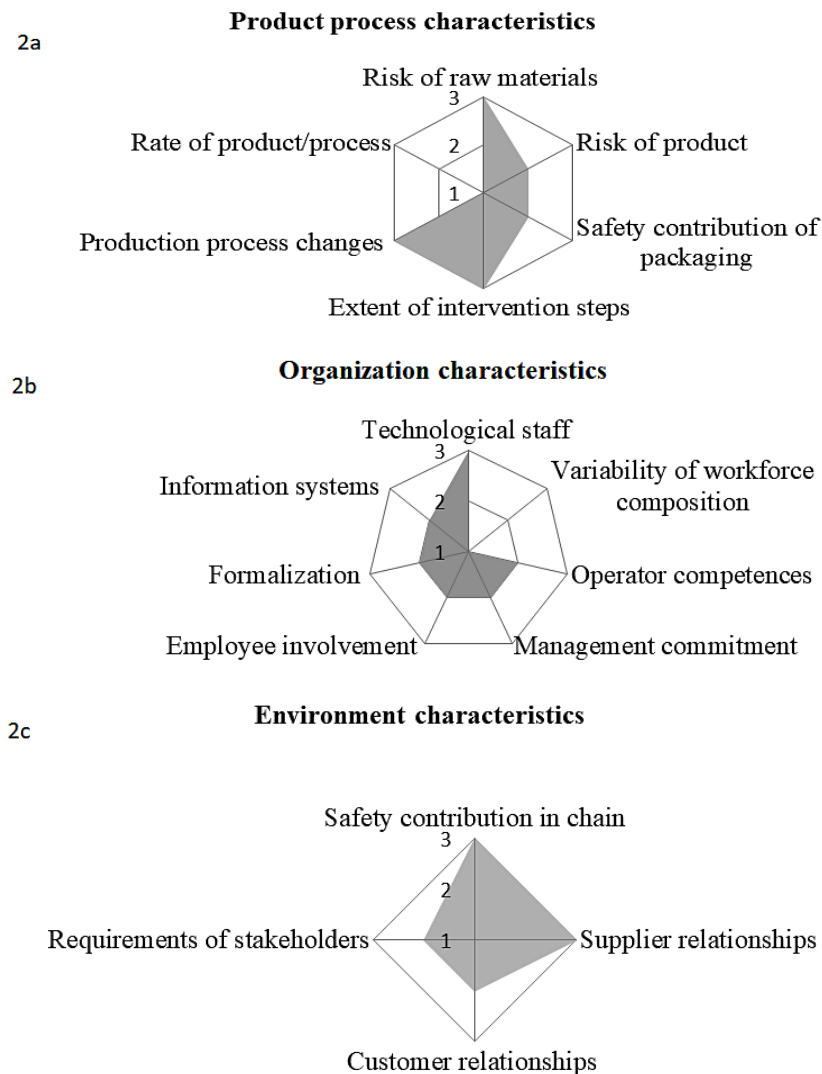


Fig.2: Results of the overall contextual situation (a-c) at the *Pangasius* fillet processing company

Results And Discussion

Self-Diagnostic Instrument

FSMS-DI is used to give an insight into the current situation of implemented FSMS through the diagnosis of *core control* and *assurance activities*, as well as the riskiness of context factors and system output with the basic assumption that the company working with riskier products and processes (context 3) need an FSMS at a more advanced level (level 3) to be able to comply with safety requirements.⁷ The structured interview was conducted to analyse the company adaptation to the contextual situation and FSMS control and *assurance activities* in order to attain good performance.

The individual results of *contextual factors* were as shown in Figure 2a-c, and the results were graded as 1, 2, or 3 which corresponded to low, moderate, or high risks, respectively. The context situation factors of the company were mainly operating at moderate risk, where 6/17 indicators were operating at high risk, 9/17 at moderate risk and 2/17 at low risk. In product process characteristics (Fig. 2a), the production process changes, the extent of the intervention process and the risk of raw materials presented a high risk (score 3). Comparing to previous studies, the same contextual situation was observed indicating that the product process characteristics of *Pangasius* fillets in Vietnam are more or less the same.^{2,3,16} The high risk in production process changes was instigated by the exposure of the product to different contamination risks during processing since some of processing steps such as filleting, washing, trimming etc. were not fully automated. The extent of intervention steps had a high risk since the frozen fillets weren't exposed to any form of inactivation or elimination of microorganisms rather the fillets were exposed to freezing process.^{6,11} The risk of raw materials was high due to the origin of *Pangasius* fish as fresh tropical water and farmed fish, with initial high microbiological counts on the skin and gills of the fish.¹⁷ The physiological characteristics of fish such as the pH (around 7), water activity (>0.98) and non-possession of any natural antimicrobial have

made it suitable for microbiological growth.^{16,18} The company has processed only one product of frozen fillet line. In addition, no packaging modification or innovative product line in the last 2-3 years was stated; as a result, the rate of product/process changes obtained a low risk (score 1).

The organizational characteristics were operated at moderate risk (Fig. 2b), different from the previous studies,^{2,3} which indicated low risk in a large company and high risk in a small company. In this company, there were 16 technological staffs and the company didn't have any quality assurance department, experts or laboratory. In the studied industry, the microbial analyses or safety controls were performed by external laboratories. The variability of workforce composition had a score of 1, indicating the workforce (low turnover of employees was longer 5 years) more stable than previous studies^{2,3} with high turnover of employees from 1-5 years.

The environmental characteristics of the studied company operated in moderate to high risk (Fig. 2c). Safety contribution provided a high risk since pathogens were not reduced to acceptable level in the production chain. The supplier relationship had a high risk due to the fact that the company didn't have its own farms; therefore, raw fish was supplied from different farms from Can Tho, Dong Thap, Ben Tre and Vinh Long province. The company gave feed to farmers but let them manage the quality on their own and only conduct antibiotic residue tests before harvesting, this may result in loss of traceability in on-farm quality-related activities.¹⁹ The requirement for stakeholders and customer relationships was operating at moderate risk (score 2) since the company had the ability to discuss product use with major critical customers but they had no influence on their FSMS. The overall mean score for all *contextual factors* was 2.2, and a score of 2 was assigned.

The control activities were less advanced since only 2/25 of the response was at level 3, whereas the majority response (11/25) was at either level 2 or level 1 and the minority response (1/25) was at level 0 (Fig. 3a-d).

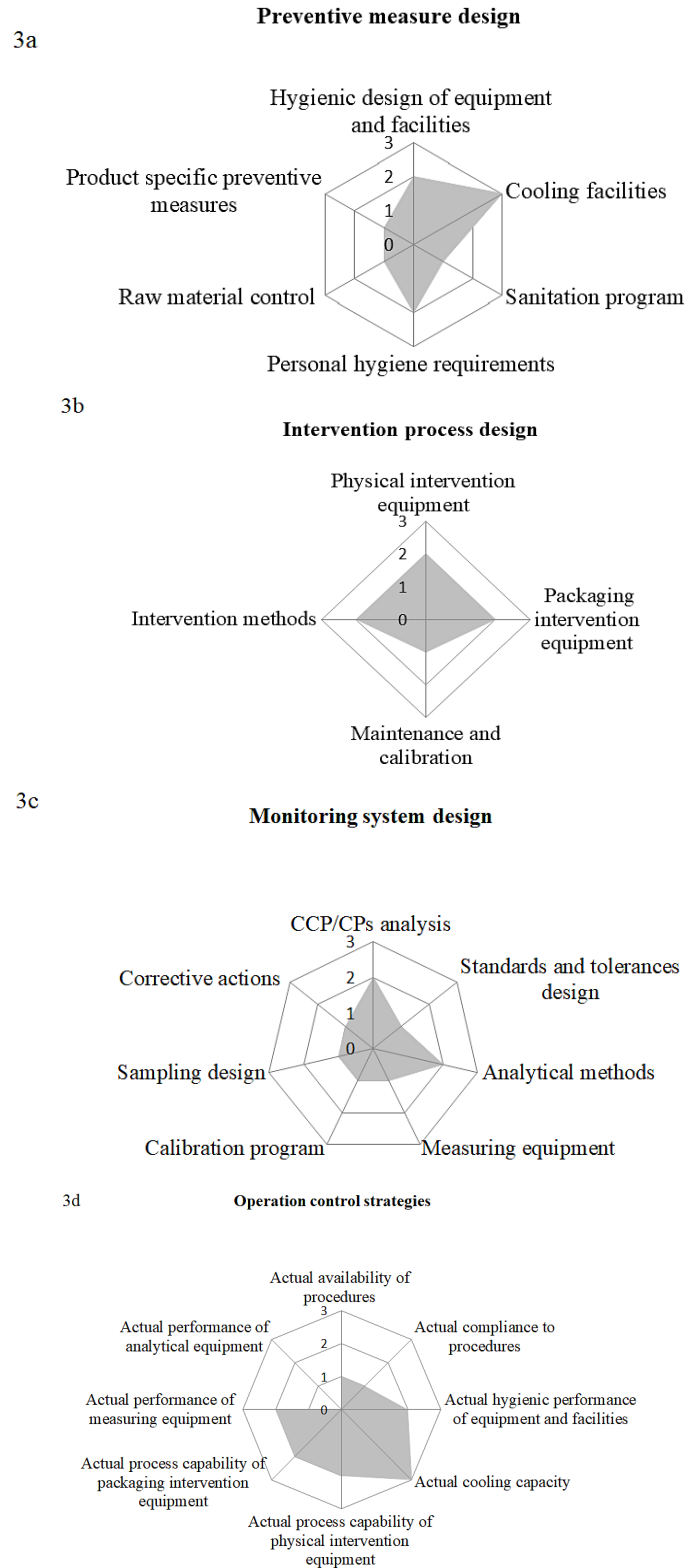


Fig. 3: Detailed results of core control activities (a-d) at *Pangasius* processing company

Preventive measures design was operating between basic to average level (Fig 3a), whereas only cooling facilities were operated at the advanced level since the interviewed company was found to have Individual Quick Freezer as well as block design freezers. Sanitation program, personal hygiene requirements, raw material control and product-specific preventive measure were operating at a basic level. Sanitation program was carried out by external commercial cleaning agents who are not specific to the production system but worked based on instructions derived from information on the label or company experience. Personal hygiene requirements were based on basic hygiene instructions. Raw material control was done based on the agreements such as the ratio of live to dead fish, disease, antibiotic residue and pesticide residue while the product-specific preventive measure was done based on company knowledge/experience and/or common knowledge. The aim of the intervention processes is to inactivate or eliminate pathogens in order to reduce them to acceptable levels.⁹ In the interviewed company, intervention process design was mainly operating at average level for physical intervention, packaging intervention and intervention methods. The company bought packaging materials from an external company which comply with standards and tolerances but not tested for own production system. Maintenance and calibration were operating at a basic level, with absence or low rate of calibration which was not well documented and initiated by problems (Fig. 3b).

The monitoring system design of the company was mainly operating at a basic level (Fig. 3c), that is program were incomplete, problem-driven, with no specific instructions, common materials and run on

an ad-hoc basis. Measuring equipment/methods were not standardised and/or not internationally acknowledged with no information/data history available. The calibration programs for measuring and analytical equipment were not clearly documented with tasks and frequency. Sampling design and measuring plans were based on experience and in-house knowledge. No information about the distribution of pathogens since the samples were taken as spot-check procedure. While standards designs were specified for critical product and process, tolerances were not clearly specified, the assessments of product/process standards were basically on historical data and company experience. Likewise, corrective actions were done based on experience, and consensus within the company with incomplete descriptions of process adjustments and handling of non-compliance products and therefore covered a score of 1.

Operation control strategies were run at basic to average level (Fig. 3d). The company had no record of the actual performance of analytical equipment (score 0), that is, there was no analytical analysis executed by the company or by external laboratories or agencies. The actual compliance with procedure obtained a score of 1 since people (food handlers) execute tasks by own insights, not aware of the existence of procedures for certain tasks whereas the actual availability of procedure was often paper-based, difficult to be understood by the users and not kept up to date. The actual hygienic performance of equipment and facilities was tested on an ad-hoc basis and regularly lead to unstable process, unexpected and unexplainable contaminations and very sensitive for minor changes.

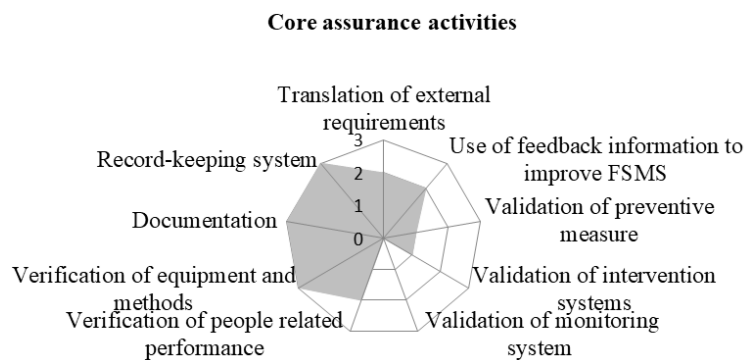


Fig. 4: Detailed results of core control activities (a-d) at *Pangasius* processing company

Core *assurance activities* in the studied company were operating at average to advanced level where 3/9 at level 3, 3/9 at level 2, 2/9 at level 1 and 1/9 at level 0 (Fig. 4). Validation of monitoring systems was given a level 0 since until the moment of this study, the effectiveness of monitoring systems has never been validated. This explains the basic level (of 1) obtained in monitoring designs. Validation of preventive and intervention systems also obtained level 1 since the effectiveness of preventive measures and intervention systems were validated on historical knowledge judged internally. Documentation and record-keeping systems were well structured and operating at advanced level, that is, activities and results were well documented, updated with assigned responsibilities, automated and available online for all, with access to external sources of information.

Different from the principle of FSMS-DI and in comparison with the contextual situation (score of 2), FSMS obtained a score of 1-2, implying that the company operated from basic to average level and at a lower level than required by the principle of FSMS-DI. Although previous studies indicate that control activities usually obtain a high score in most of the food processing companies,^{8,20,21} in this study the control activities had a lower score (1-2) whereas the *assurance activities* had a score of 2. This means that the company is working on providing confidence to stakeholders about meeting the requirements than the practical application in keeping (control) of product properties, production processes and human practices within certain acceptable tolerance limits.

The *food safety performance indicator* (FSPI) gives insight into food safety level of the food products designed by the authors.⁵ FSPI included seven indicators used to establish food safety performance of the company. Four levels (0, 1, 2 and 3) were defined referring to no indication of food safety performance; absent/not measured, poor performance; minimum follow-up, moderate performance; standard follow-up and good performance; comprehensive system evaluation, respectively. There was no indicator attained either the highest level 3 or the lowest level 0, 6/7 of the analysed performance indicators

obtained level 2 and 1/7 obtained level 1. The food safety performance was assigned a score of 2. Generally, the FSMS-DI was able to provide the first indication of the microbiological safety of the company through the evaluation of its core control and core assurance. The contextual situation didn't fit well with the FSMS activities of the company. The FSPI shows that the company is operating at average safety level. The FSMS-DI was able to indicate the weak and strong points of the company in relation to actual food safety output.

Microbial Assessment Scheme

The food safety output was assessed by MAS which comprises effective microbiological analysis to give an insight into the contamination profiles and the distribution of microbial contamination. A total of 117 samples of *Pangasius* fillets, water and food contact surfaces were collected and analysed to understand the microbial distribution in the selected critical sampling location during the processing of *Pangasius* fillets and from it, the microbial safety score was assigned.

The differences in mean values of total mesophilic count (TMC), *E. coli*, *Coliform* and *S. aureus* during the three different visits and three independent sampling times were analysed to understand the variability of microorganisms (Fig. 5). The total mesophilic count and *E. coli* didn't show any significant difference in three different visits ($p > 0.05$). However, there was a significant difference ($p = 0.036$) in the *Coliform* between the first and the third visit. Likewise, *S. aureus* in *Pangasius* fillets showed significance difference ($p = 0.018$) between the first and the second visit. This reflects the difference in the hygienic practices and process control on different days and that uniformity in product/process changes are not attained. The actual compliance to procedures, hygienic performance of equipment and the actual performance of measuring equipment could also contribute to variation in product/process and affect the overall quality of the final product.¹⁰ The total count of TMC, *E. coli*, *Coliform* and *S. aureus* did not differ significantly ($p > 0.05$) in three independent samplings.

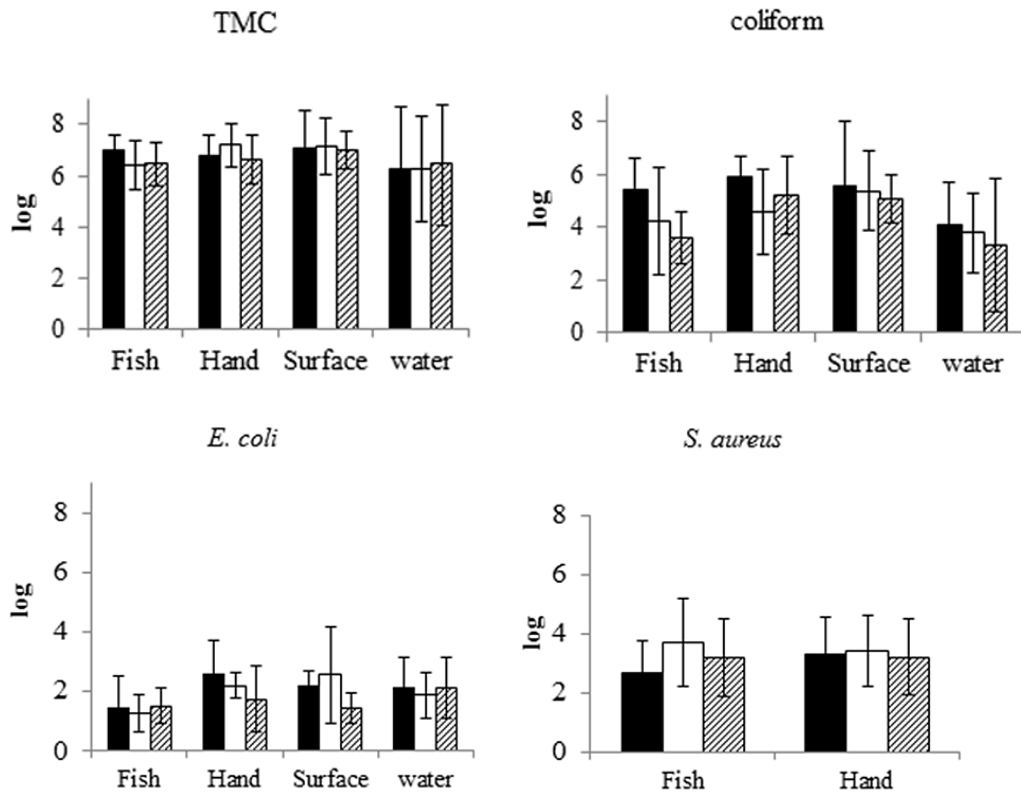


Fig. 5: Dynamics of microorganisms among visits, visit 1 (■), 2(□) and 3 (▨)

Microbial Quality and Safety Fillets at *Pangasius* Processing Company

Fish and fishery products are frequently contaminated with aerobic enteropathogens from contaminated water during culturing or from poor personnel hygiene during processing and distribution activities.^{22,23} Microbial profile of fish as influenced by bacterial ecology of *Pangasius* fish, personal hygiene, processing equipment, water used during processing was observed. Total mesophilic count, *E. coli*, *Coliform* and *S. aureus* in different processing steps showed a significant increase from raw fish to trimming process, and then slightly decrease to packaging step (Table 2). The same trend was observed on food contact surfaces and hands of food operators (Table 2). There was a significant increase ($p = 0.0$) in the TMC from raw fish (5.8 ± 0.7 log CFU/g) to frozen-packed fillets (7.2 ± 0.3 log CFU/g), whereas the range of <2.0 - 8.6 log CFU/ml, 5.1 - 9.1 log CFU/100cm² and 5.3 - 8.5 log CFU/100cm² was observed in water, food contact surfaces and hands/gloves of food handlers respectively. High

TMC count is often associated with an increased number of spoilage bacteria and has been used to estimate the freshness of fish.^{24,25} However, some studies indicated that TMC cannot always be used to give a realistic estimation of the microbial contamination levels especially in frozen or chilled food.^{18,26} Likewise, there was a significant increase ($p=0.014$) in *S. aureus* from raw fish (1.2 ± 0.4 log CFU/g) to trimming fillets (3.4 ± 2.2 log CFU/g). High counts of *Coliform*, *E. coli* and *S. aureus* on fish samples were observed during filleting and trimming steps and were widely distributed in water, food contact surfaces and hands of food operators (Table 2). The examined counts of *E. coli*, *Coliform* and *S. aureus* were mainly influenced by poor quality control activities such as personal hygiene practices, sanitation programs, product-specific preventive measures and raw material control as a result of water contamination.²⁷ The microbial counts from this company (ca. 100 tons/day) exceed the total counts observed in small-scale production of 35 tons/day² and a large scale production of 200 tons/day.³

Table 2: Detailed distribution of selected microorganisms in different sampling locations

| Type of sample (n=117) | Process | Sampling Location (SL) | Frequency of sampling | Quantitative results (mean ± std, Range) | | Qualitative results | | | | |
|--|----------------|------------------------|--------------------------|--|----------------------------|---------------------------|--|-------|-------|-------|
| | | | | TMC | <i>E. coli</i> | <i>S. aureus</i> | <i>L. monocytogenes</i> <i>V. cholerae</i> <i>Salmonella</i> | | | |
| Fish (CFU/g) | Raw fish (FR) | 1 | 3 × 3 | 5.8 ± 0.7 (4.1 - 6.5) | 1.4 ± 0.7 (<1 - 2.3) | 4.1 ± 1.5 (2.0-5.6) | 1.2 ± 0.4 (<2.0 - 3.1) | 9-Jan | 9-Aug | 0/9 |
| | Filleting (FF) | 3 | 3 × 3 | 6.4 ± 0.9 (5.0 - 8.0) | 1.1 ± 0.4 (<1.0 - 2.1) | 5.2 ± 1.5 (2.0 - 6.4) | 1.1 ± 0.4 (<2.0 - 4.6) | 9-Feb | 9-Sep | 9-Jan |
| | Trimming (FT) | 7 | 3 × 3 | 7.1 ± 0.4 (6.6 - 7.6) | 2.1 ± 1.1 (<1.0 - 4.4) | 5.1 ± 0.9 (4.1 - 6.6) | 3.4 ± 2.2 (<2.0 - 6.2) | 9-May | 9-Sep | 0/9 |
| Water (CFU/ml) | Packaging (FP) | 11 | 3 × 3 | 7.2 ± 0.3 (6.8 - 7.7) | 1.1 ± 0.2 (<1.0 - <2.0) | 4.9 ± 1.4 (<1.0 - 6.3) | 1.7 ± 2.3 (<2.0 - 4.6) | 9-Jul | 9-Sep | 0/9 |
| | Bleeding (WB) | 2 | 3 × 3 | 8.1 ± 0.4 (7.4 - 8.6) | 2.9 ± 0.6 (2.0 - 3.7) | 4.8 ± 1.0 (3.5 - 6.3) | NA | 9-Jan | NA | 9-Mar |
| | Washing1(WW1) | 5 | 3 × 3 | 7.2 ± 0.5 (6.4 - 7.9) | 2.1 ± 0.7 (<1.0 - 2.8) | 5.3 ± 0.5 (4.5 - 5.9) | NA | 9-Jan | NA | 9-Jan |
| Hand (CFU/100 cm ²) | Glazing (WG) | 10 | 3 × 3 | 3.6 ± 1.7 (<2.0 - 5.2) | 1.2 ± 0.6 (<1.0 - 2.7) | 1.7 ± 0.8 (<1.0 - 2.8) | NA | 0/9 | NA | 0/9 |
| | Filleting (HF) | 4 | 3 × 3 | 6.9 ± 0.7 (5.3 - 7.5) | 1.9 ± 0.6 (<1.0 - 3.2) | 5.4 ± 0.7 (4.1 - 6.1) | 1.7 ± 0.5 (<2.0 - 4.1) | 0/9 | 6-May | 9-Jan |
| | Trimming (HT) | 8 | 3 × 3 | 7.7 ± 0.3 (7.3 - 8.2) | 2.9 ± 1.2 (<1 - 5.4) | 6.5 ± 0.6 (4.1 - 7.3) | 3.9 ± 1.8 (<2.0 - 5.9) | 9-Jun | 6-Jun | 0/9 |
| Contact surface (CFU/100 cm ²) | Packaging (HP) | 12 | 3 × 3 | 6.4 ± 0.9 (5.4 - 8.5) | 1.7 ± 0.5 (<1.0 - <2.0) | 3.6 ± 1.2 (<2.0 - 5.6) | 2.4 ± 0.7 (<2.0 - 4.4) | 0/9 | 6-May | 0/9 |
| | Skinning (CS) | 6 | 3 × 3 | 7.7 ± 0.3 (6.8 - 8.3) | 2.6 ± 1.7 (1.8 - 6.9) | 6 ± 0.9 (4.2 - 7.2) | NA | 8-May | NA | 0/9 |
| | Trimming (CT) | 9 | 3 × 3 | 7.8 ± 0.7 (7.1 - 9.1) | 2.0 ± 0.7 (<1.0 - 3.5) | 6.8 ± 0.9 (5.4 - 8.5) | NA | 9-Jul | NA | 0/9 |
| Packaging (CP) | 13 | 3 × 3 | 5.8 ± 0.4 (5.1 - 6.4) | 1.7 ± 0.5 (<1.0 - 2.0) | 3.5 ± 1.0 (<2.0 - 4.7) | NA | 9-Jan | NA | 0/9 | |
| Food safety level | | | | 1 | 2 | 1 | 2 | 1 | 1 | 2 |

L. monocytogenes were identified as natural microflora of aquatic systems due to the ability to survive outside host organisms for a long time.^{28,29} In this study 15 out of 36 collected fish samples were *L. monocytogenes* positive. The distribution of *L. monocytogenes* was observed in all fish samples from raw fish, filleting, trimming and packaging, these results were the same as those observed by the researchers³⁰ at catfish fillets processing company. *L. monocytogenes* had the ability to form biofilms as well as surviving under refrigeration conditions, low pH and high salt concentration.^{31,32} This explains the high prevalence observed in food contact surfaces where 13/26 samples were *L. monocytogenes* positive. A study³³ reported that floor drains in food processing facilities are typical sites for persistent *Listeria spp.* and maybe a source of contamination in the processing plant, environment and possibly in food products. A study conducted in the fishing vessel and fish factories, indicated contamination of food surface before the fish entered the vessel.²⁵ Likewise, *L. monocytogenes* were tested positive in 6/9 during trimming steps on the hands/gloves of food operators. The previous study reported that there was consistently higher occurrence (0.2–3.9%) of *L. monocytogenes* in fish and fishery products

during processing as well as the highest incidence in ready to eat products (6%).³⁴ Meanwhile, studies documented the presence of *L. monocytogenes* in the tropical environment and therefore fish itself may be a vehicle³⁵ and subsequently act as a source of contamination to processing facilities during evisceration, skinning and trimming.³⁶ Therefore, the poor hygienic conditions and low frequency of cleaning and disinfection (mainly done at the end of each processing day observed during sampling) were suggested to be the main reasons for the pervasiveness of *L. monocytogenes*.

V. cholerae was found in 35 out of 36 fish samples collected. *V. cholerae* is naturally present and widely distributed in the aquatic environments and it has been shown that fish contains several strains of *Vibrio spp.* in their digestive tracts.^{23,37} A high prevalence of *V. cholerae* was also observed in *Pangasius* fillets marketed in Poland, Germany and Ukraine.²⁷ *V. cholerae* was found in 16 out of 18 samples on hand/gloves of food operators. The transmission route of *V. cholerae* found in fish samples was proposed to come from gastrointestinal parts during filleting and from food handlers with water as the main vehicle for transmission.^{2,38}

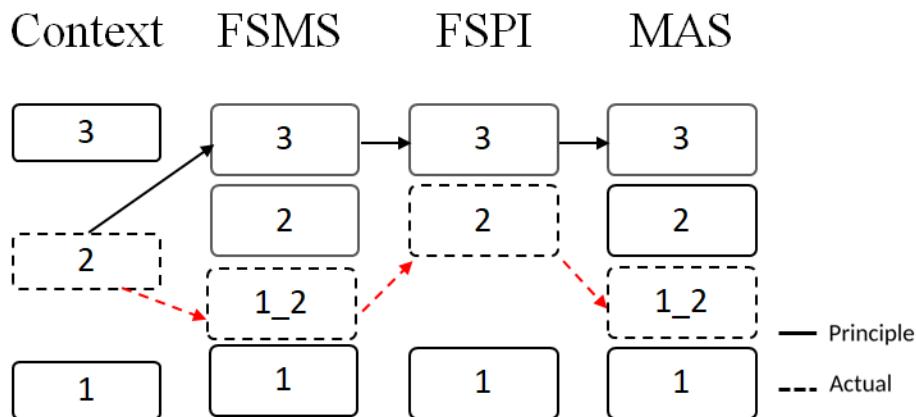


Fig. 7: Principle versus actual FSMS performance at *Pangasius* fillet processing company F

Salmonella spp. was isolated from 1/36 fish samples in the filleting step, in 1/27 hand samples and in 4/27 water samples. Although some previous studies have indicated that *Salmonella spp.* aren't present in the aquatic environment, can be introduced through animal or human faecal contamination and

sewage pollution, or cross-contamination during transportation or storage, making seafood carriers for *Salmonella spp.*³⁹⁻⁴¹ The study conducted at two Tilapia sashimi processing plants in Taiwan stated that inadequate sanitation may be the main route for *Salmonella spp.* in fish processing companies.⁴²

Surprisingly, the lowest microbial counts tested were seen in the raw fish samples whereas these counts increased in the subsequent processing steps (i.e. filleting, trimming and packaging) (Table 2). This indicates the uncontrolled contamination throughout processing since fish fillets are assumed sterile while the high microbial counts observed may come from the intestinal parts, skin and gills of the raw fish in filleting, then spreading out to subsequent processing steps.¹⁷ In the filleting step, a wide range was observed in the TMC and *Coliform* on fillet samples (5.0-8.0 and 2.0-6.4 log CFU/g, respectively). Pathogens including *V. cholerae*, *L. monocytogenes* and *Salmonella spp.* were detected in 9, 2 and 1 out of 9 fish samples, respectively during filleting (Table 2). The filleting step may be one of the critical routes for contamination as a result of gut perforations. Previous studies have reported that most microorganisms isolated from fillet samples were endogenous of gills or intestinal tracts of farm-raised freshwater fish.^{18,43} Particularly, the microbial counts of TMC, *E. coli*, *Coliform* and *S. aureus* (7.1 ± 0.4 , 2.1 ± 1.1 , 5.1 ± 0.9 and 3.4 ± 2.2 log CFU/g, respectively) were the highest in the trimming step. *L. monocytogenes* and *V. cholerae* were positive whereas *Salmonella spp.* was absent in all trimmed fillets.

The trimming step can be the main source of contamination as the manual operation can induce the contamination from hands, food contact surfaces into the trimmed fillets in the company sampled. Hence, these results suggest that sanitation programs, personal hygiene requirements, frequency of cleaning and disinfection and automation of the process should be set-up. It is suggested that the study on biofilm formation and effectiveness of cleaning and disinfection in the trimming step will further carry out. In the packaging step, the TMC and *Coliform* in the frozen final products were 7.2 ± 0.3 and 4.9 ± 1.4 log CFU/g, respectively (Table 2) and these microbial counts exceeded the recommended guidelines and criteria (Table 1). The observation in Table 2 shows a decrease in *Coliform* and *S. aureus* count (ca. 1.5 log CFU/g) after freezing. The reduction in microbial counts can be due to the effect of freezing temperature. The findings from this study did not in line with the previous study⁴⁴ that reported that gram-negative bacteria die more rapidly during

frozen storage than gram-positive. The presence of pathogens i.e. *L. monocytogenes* (7/9) and *V. cholerae* (9/9) did not comply with the recommended guidelines and criteria. Therefore, the producers should take into account the hygienic practices especially in the final packaging step as this is the product that goes to customers. Although *Pangasius* fillets are to be cooked before consumption, the presence of pathogenic bacteria in the final product reflects a failure in core control and *assurance activities* of the processing company.^{6,10}

Overall, the total score from tested microbial parameters was 10/21 (Table 2) indicating a food safety output with an assigned score of 1_2. It means that the sampled company was operating at low-moderate performance FSMS and some improvements in sanitation programs, personal hygiene requirements, raw material control and product-specific preventive measures, sampling designs, analytical methods and corrective actions are emphasized.^{11,45}

The assigned scores of FSPI (score 2) were compared with the assigned scores from MAS (1_2) in order to validate if the scores selected FSPI and the diagnosis provided a realistic indication of the microbial performance of an implemented FSMS-DI. This was contributed by the fact that the studied company imbue in core *assurance activities* operated at moderate level than core control activities which operated at low to moderate level by means of evaluating by their own company. In addition, the FSMS-DI was a qualitative assessment while MAS was a quantitative and actual assessment of the performance FSMS.

Conclusion Remarks

The results from FSMS-DI provided an insight into the general profile of FSMS in the studied company. The results of core control and *assurance activities* didn't fit well with the contextual situation of the company. Although the FSPI showed that the performance FSMS was operating at moderate safety level whereas it was poor to moderate level by microbial assessment scheme. The high distributions and variations in the microbial counts in different visits demonstrate that the microbiological contamination is not under control and the current

FSMS is not performing well. Improvement is emphasized in general control of raw material, personal hygiene practices as well as cleaning and sanitation programs with respect to products quality as identified to be the major source of cross (contamination). The HACCP system in place should be revised and improved to meet HACCP requirements for the certification in hold. The overall evaluation of the company FSMS by the internal or external assessment yearly is advised. These findings can be used by the studied company and other related companies to improve their FSMS as well as HACCP systems in place.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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