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ORIGINAL ARTICLE

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Evaluation of the microbiological safety and quality of Vietnamese *Pangasius hypophthalmus* during processing by a microbial assessment scheme in combination with a self-assessment questionnaire

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Abstract *Pangasius hypophthalmus* (referred to as *Pangasius* or tra fish in Vietnam) has become highly appreciated by consumers in the European Union, USA, Japan, etc. and is of worldwide economic importance. Therefore, the microbial quality and safety of *Pangasius* fish processed for export was assessed by means of a microbial assessment scheme throughout the entire production process. A total of 144 samples were collected from various processing steps and analyzed. It was determined that the microbial safety and quality of the products was not guaranteed as the contamination levels remained high throughout processing. *Escherichia coli, Staphylococcus aureus* and *Vibrio cholerae* were present on the hands of food operators, particularly those in the packaging area. Moreover, the presence of *Listeria monocytogenes* (1 positive out of 9 samples) and *V. cholerae* (4/9) on the final products was likely a result of inadequate hygiene practices in the processing environment. Also discussed in this paper are the results of a self-assessment questionnaire, which provide insight into the performance of the food safety management system currently implemented at the company. These data are of major importance in order to contribute valuable information to the local and international trade point of view in general and to the intended customers in particular.

Keywords Tra fish · *Pangasius hypophthalmus* · Food safety management systems · Microbial safety · Microbial quality

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Introduction

Aquaculture products are a healthy source of protein combined with a low fat content. They account for 30 % of all fish and shellfish production [1]. In recent years, Vietnamese aquaculture production has increased steadily, reaching 5.74 million tons in 2012 [2]. Tra fish *Pangasius hypophthalmus,* a farmed freshwater fish, has become an economically valuable product for Vietnam in recent years. For instance, Vietnam is the biggest *Pangasius* exporter to the US and Vietnamese *Pangasius* were ranked the sixth favorite fish species in the US in 2011 [3]. Besides the US, the Vietnamese tra fish are exported to over 80 countries worldwide including those in the European Union, Canada, Japan, etc. who demand high microbial quality and safety standards [4, 5].

Although Vietnamese *Pangasius* is accepted as being of good nutritional quality and safety [4], the control of (cross) contamination by pathogens is still challenging. Pathogenic bacteria can be transmitted into aquaculture products during rearing, handling and processing as a result of improper hygienic conditions. For example, *Salmonella* spp., *Vibrio cholerae* and *Listeria monocytogenes* originate from rearing ponds or the processing environment [6]. With regards to the contamination of Vietnamese aquaculture products with pathogens, a very high prevalence of *Salmonella* (32 %) was detected on fish products from Vietnam [7]. This prevalence was much higher than imported products tested from Indonesia (10–20 %), the Philippines (10–20 %), India (10–20 %), Thailand (8 %), and Mexico (8 %). Moreover, Vietnamese *Pangasius* products destined for European countries have been rejected in the past due to the presence of bacteria of public health concern such as *Salmonella* spp. and *Listeria monocytogenes* and high counts of *Escherichia coli* (100–4,900 CFU/g) [8]. For example, according to RASFF [8] there were 20 cases of rejection between 2010 and 2012. Tong Thi et al. [9] emphasized that not only very high counts of presumptive Enterobacteriaceae but also the presence of several pathogenic species of Enterobacteriaceae (i.e. *Providencia alcalifaciens, Shigella flexneri* and *Klebsiella pneumoniae*) occurred on *Pangasius* products from small-scale processing plants (35 tons/day) in Vietnam. However, there are still very few data on the transmission routes of pathogenic bacteria during the handling of *Pangasius* products. Moreover, data on the microbial safety of *Pangasius* products would in general provide valuable information for a local and international trade point of view, and for the intended customers in particular. In fact, to date only one study has indicated that the microbial safety risk was medium high in processed Vietnamese *Pangasius* fish [10].

The Vietnamese fishery industry has dedicated much effort and resources towards satisfying not only the Vietnamese regulations but also international standards. Recently, over 100 Vietnamese companies which process *Pangasius* products for export had implemented good manufacturing and hygienic practices (GMP and GHP), hazard analysis critical control point (HACCP), and/or other food quality or food safety management systems (FSMS) such as BRC, IFS, ISO 9001, ISO 14001, etc. [11]. Despite the large economic value of *Pangasius* products to Vietnam, little research has been conducted on the performance of FSMS of *Pangasius* processing companies and their influence on microbial quality and safety during processing. Previously, some studies emphasized that different food processing plants can deal with different microbial loads and food safety issues due to variability in implementing and understanding of the performance of FSMS [12]. To know whether FSMS are implemented adequately, the number of microorganisms and variation of microbial counts can be assessed throughout the processing chains by means of a microbial assessment scheme (MAS) [13]. The microbial assessment scheme is a vertical microbial sampling plan throughout the production process, from raw materials to final product. Such a microbial sampling plan has previously been applied to gain insight into the production processes of various types of foods i.e. ready-to-eat meals by Daelman et al. [14]; pork meat industry by Jacxsens et al. [13]; catering services by Lahou et al. [15]; and poultry slaughterhouses by Sampers et al. [16]. To date only one study has been applied to *Pangasius* fish produced in a large-scale Vietnamese company processing 200 tons daily [10]. However, most Vietnamese companies processing *Pangasius* are actually of small-medium size (<100 tons *Pangasius* processed per day).

In this study, the FSMS currently implemented at a small-scale plant processing Vietnamese tra fish was evaluated by means of a MAS throughout the entire production process. In addition, assessment of the context, control and assurance activities, and food safety output of the FSMS applied was performed by a self-assessment questionnaire.

Materials and methods

Characterization of the sampled company

Processing plant

The company evaluated in this study is located in Can Tho City, Vietnam, and has a daily production capacity of ca. 35 tons. Approximately 300 people work in the processing area of the company. Food safety and/or quality management systems such as HACCP, BRC and IFS are applied at the company. The *Pangasius* products of the company are mainly exported to European countries (i.e. the UK and Greece), the United Arab Emirates, Egypt, Thailand, etc.

Process flow chart

The flow chart for the processing of *Pangasius* fillets at the company evaluated is shown in Fig. 1. The fish was transported from suppliers to the factory by van within 30 min. Upon arrival at the company, bleeding and filleting was done manually followed by washing in a continuous flow of water. The fillets were then skinned mechanically. After washing in potable water, the subcutaneous fat and red muscle on the surface of the fillets was scraped off with

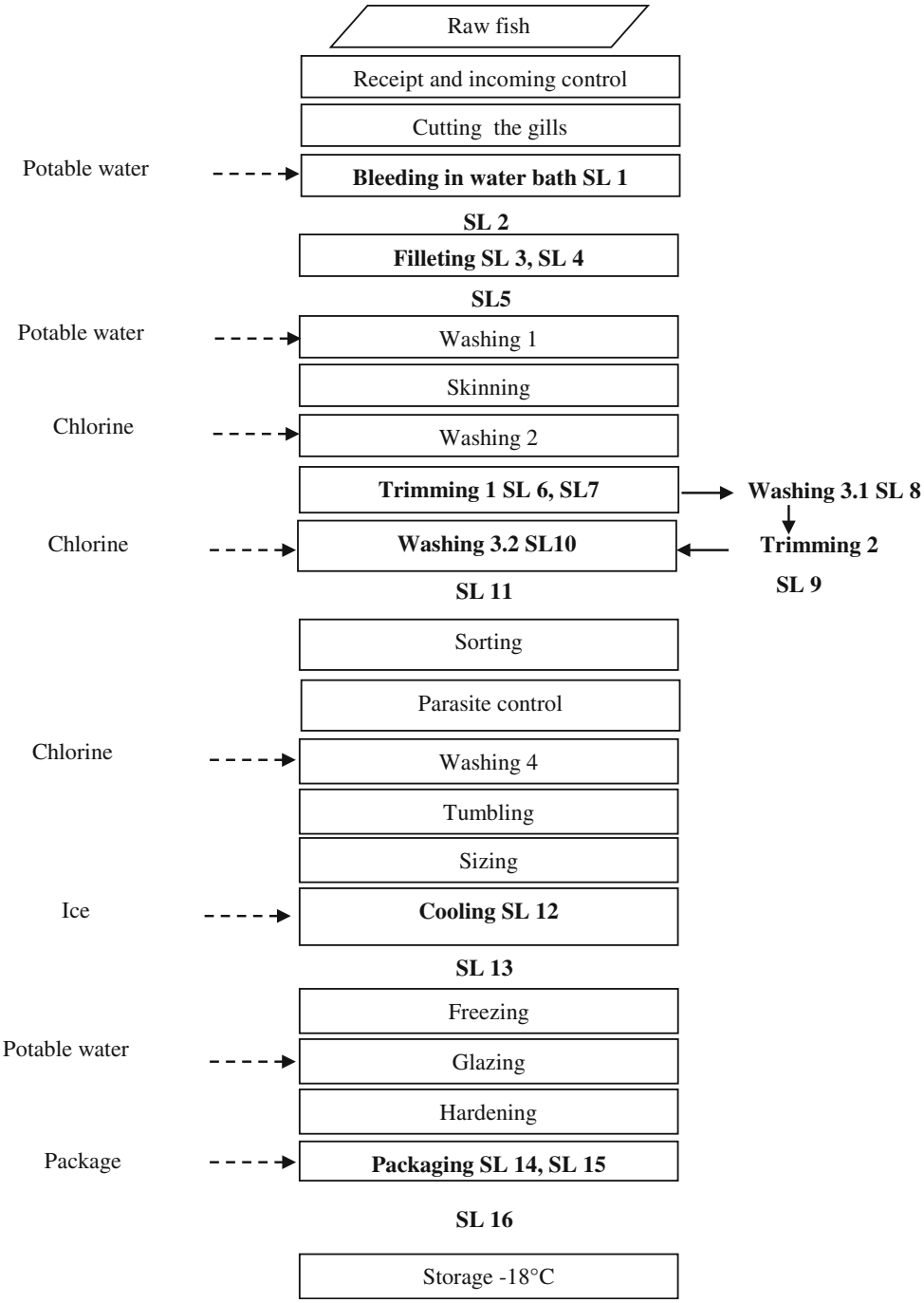

Fig. 1 Flow chart of production process of Vietnamese *Pangasius* (*Pangasius hypophthalmus*) in a small-scale company. Sampling locations (SL) are indicated in *bold*

a knife during the first trimming step. The fillets were then washed in potable water before the second trimming step. During this step a knife was used to trim the edges of fillets. Thereafter the fillets were washed manually in a water-bath with potable water containing 50 ppm of chlorine. The fillets were then sorted manually based on color into white, pink to red, and yellow groups. Every fillet was checked for (putative) parasites by placing them on a translucent table illuminated from below. Thereafter, water treated with 50 ppm of chlorine was used to wash the fillets. The fillets were then treated with unspecified additives in tumblers for 1 h. The fillets were then graded manually according to weight into four groups: 60–120, 120–170, 170–220 and ≥220 g per fillet. Before freezing, the fillets (5 kg) were placed into plastic bags and cooled with flake ice. During the freezing process, the individual fillets were manually placed into an individual quick freezer (IQF) and frozen until a core temperature of −18 °C was achieved. The frozen products were packed into carton boxes, labeled and stored at −18 °C.

Sampling locations (SL)

The samples collected in this study consisted of both fish fillets and environmental samples i.e. water and surface, hand or glove swabs. The points at which sampling was done are shown in Fig. 1 (SL 1–SL 16). The sampling locations included raw materials, production processes like filleting, trimming and cooling and intervention steps such as washing in water baths and packaging.

Sampling frequency

The company was visited 3 times during a 4-week period (week 10–13) in March 2013. The samples were collected at all 16 SLs at three different times (ca. 8 a.m., 12 a.m. and 2 p.m.) during each visit (Table 1). A total of 144 samples were collected, including 54 *Pangasius* samples, 36 swabs of hands or gloves, 27 swabs of food contact surfaces and 27 water samples.

Table 1 Overview of the microbiological parameters investigated at each sampling location

Samples	Number	Sampling locations	Investigated parameters
Water	1	Bleeding	Total aerobic psychotrophic count (TPC)
	8	Washing 3.1	
	10	Washing 3.2	
			E. coli
			Coliforms
			Listeria monocytogenes
			Salmonella spp.
			Vibrio cholerae
Fish	2	After bleeding	TPC
	5	After filleting	*E. coli*
	9	After trimming	Coliforms
	11	After washing 3.2	*S. aureus*
			Listeria monocytogenes
	13	After cooling	*Salmonella* spp.
	16	After packaging	*Vibrio cholerae*
Hands or gloves	3	Filleting	TPC
	6	Trimming	*E. coli*
	12	Cooling	Coliforms
	14	Packaging	*S. aureus*
			Listeria monocytogenes
			Salmonella spp.
			Vibrio cholerae
Food contact surfaces	4	Filleting	TPC
	7	Trimming	*E. coli*
	15	Packaging	Coliforms
			Listeria monocytogenes
			Salmonella spp.

Sampling and analysis method

Sampling

The overall microbial quality i.e. total aerobic psychrotrophic counts, hygiene indicators (*E. coli*, and coliforms), personal hygiene indicators (*Staphylococcus aureus*) and pathogens (*Listeria monocytogenes*, *Salmonella* spp. and *Vibrio cholerae*) were determined depending on the type of microbial samples (Table 1).

For the fish samples, a *Pangasius* fillet (ca. 200 g) was aseptically taken with sterile tweezers and placed in a Stomacher bag. For food contact surfaces and the hands or gloves, swabs were taken vertically, horizontally and diagonally on a 100 cm^2 surface. Before swabbing, sterile swabs (Copan, Italy) were pre-moistened in 5 ml maximum recovery diluent (MRD, Merck, Darmstadt, Germany) for enumeration of total aerobic psychrotrophic counts, *E. coli*, coliforms and *Staphylococcus aureus*; in 5 ml Demi-Fraser broth (Merck, Darmstadt, Germany) for detection of *L. monocytogenes*; in 5 ml buffered peptone water (Oxoid, Basingstoke, UK) for detection of *Salmonella* spp.; and in 5 ml alkaline saline peptone (Merck, Darmstadt, Germany) for detection of *V. cholerae*. Every moistened swab was applied to each food contact surface, and then inserted back into its tube containing 5 ml of solution.

With regards to the water samples, ca. 500 ml of water from three different locations in the water baths was collected into sterile Stomacher bags. Thereafter, 1 ml of water was aseptically taken for microbial analyses. All samples were taken aseptically, stored in ice and transported in insulated boxes to the Laboratory of Microbiology and Biotechnology (Department of Food Technology, Can Tho University, Vietnam) for microbial analyses within 24 h of sampling.

Quantitative microbial analysis

Upon arrival in the lab, ca. 25 g of sample was aseptically taken from different parts of a fillet by means of sterile scalpels and tweezers and placed in a sterile Stomacher bag. To this, 225 ml of sterile MRD was then added, after which the mixture was homogenized for 1 min in a Stomacher. For water samples, 1 ml of water was aseptically transferred to 9 ml of MRD. The water samples (and also the swab samples in MRD) were vortexed for 10 s. Thereafter, a tenfold serial dilution series was performed. The total psychrotrophic counts were determined on Aerobic Count Plate petrifilms^{TM} (3M^{TM} Microbiology Products, St. Paul, MN, USA) following incubation at 22 °C for 72 h. *E. coli* and coliforms were enumerated on *E. coli*/coliform petrifilms^{TM} (3M^{TM} Microbiology Products, St.

Paul, MN, USA) after incubation at 37 °C for 48 and 24 h, respectively. *S. aureus* was enumerated on Staph Express Count petrifilm™ (3M™ Microbiology Products, St. Paul, MN, USA) following incubation at 35 °C for 24 h.

Qualitative analysis

Presence of *L. monocytogenes*

For fish samples, 25 g of sample was added to 225 ml of Demi-Fraser broth. For water samples, 1 ml was transferred to 4 ml of Demi-Fraser broth. The fish, water and swab samples in Demi-Fraser broth were then pre-enriched by incubation for 24 h at 30 °C. Subsequently, 0.1 ml was inoculated in 10 ml of Fraser broth solution (Merck, Darmstadt, Germany) and incubated for 48 h at 37 °C. This culture was then streaked on ALOA (Agar Listeria Ottaviani Agosti, Biolife, Milan, Italy) for 48 h at 37 °C incubation: typical colonies of *L. monocytogenes* are a green–blue color, surrounded by an opaque halo.

Presence of *V. cholerae*

For the fish samples, 25 g were first transferred to 225 ml alkaline saline peptone water (pH = 8.6). Pre-enrichment was done by incubating for 6 h at 41.5 °C, with the exception of the deep-frozen samples obtained from SL 16, which were incubated at 37 °C. From each water sample, 1 ml was transferred to 4 ml of alkaline saline peptone and also incubated for 6 h at 41.5 °C. Subsequently, 1 ml of the pre-enriched sample cultures were inoculated into 10 ml of alkaline saline peptone water and incubated for 18 h at 41.5 °C. A loopful of the second culture was then streaked onto the surface of thiosulfate citrate bile salts sucrose (TCBS) agar plates (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. Thereafter, typical colonies (yellow and smooth colonies) were inoculated on tryptone soya agar (TSA, Oxoid, Hampshire, UK) supplemented with 1.5 % of NaCl (Merck, Darmstadt, Germany) for 24 h at 37 °C for confirmation. Confirmation was on the basis that *V. cholerae* is Gram negative and oxidase positive.

Presence of *Salmonella* spp.

The pre-enrichment of the fish (25 g of fish in 225 ml of buffered peptone water), water (1 ml of water sample in 4 ml of buffered peptone water) and swab samples (in buffered peptone water) was performed by incubation at 37 °C for 18 h. Following pre-enrichment, 0.1 ml of the first pre-enrichment culture was transferred to 10 ml of Rappaport Vassilliadis soya peptone broth (RVS, Oxoid, Basingstoke, UK). The inoculated RVS tubes were then incubated for 24 h at 41.5 °C. A loopful of culture from the RVS tubes was streaked onto xylose lysine deoxycholate agar (XLD, Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h. Thereafter, typical *Salmonella* colonies were picked from the XLD plates and transferred to XLD slants. They were transported in this form to the Laboratory of Food Microbiology and Food Preservation (Ghent University, Belgium) for further phenotypical and serological confirmation tests.

Self-assessment questionnaire on food safety management system

A questionnaire with 58 indicators was designed based on the work of Luning et al. [17, 18] and Jacxsens et al. [19, 20]. The questions were categorized under the following topics: (a) context factors (i.e. product characteristics, production process, organization, and chain environment), (b) control activities (i.e. preventive measures, intervention processes, monitoring system design and their operation), (c) assurance activities (i.e. setting system requirements, validation, verification activities, documentation and record-keeping), and (d) food safety performance. The questionnaire was answered by the people responsible for quality assurance (QA) at the company via an in-depth interview (ca. 3 h).

Of the 58 questions, 17 assessed the context factors, 25 assessed the control activities, 9 assessed the assurance and 7 assessed the food safety performance. The 17 questions on the context were graded as situation 1, 2, or 3 which corresponded to low, potential, or high vulnerability (to safety problems), ambiguity (lack of insight in underlying mechanisms), and uncertainty (lack of information), respectively [18]. For the 25questions on the control activities, four levels were defined (0, 1, 2 or 3), which corresponded to not relevant, incomplete, guidelines-based and science-based/fit-for-purpose, respectively. The nine questions about assurance activities also comprised four levels (0, 1, 2 and 3), with unknown, historical knowledge (but no analysis), restricted and comprehensive levels, respectively [17]. All seven questions about food safety performance were defined at four levels (0, 1, 2 and 3) referring to absent/not measured, minimum follow-up, standard follow-up and comprehensive system evaluation, respectively [20].

For each question, the interviewees had to select which situation or activity level was the most representative for their company. Each question was well defined and designed by "if then" combined with supportive information to guide the interviewees in advance during the interview.

Interpretation of results

A comparison was made between the microbial counts observed on fish samples and the tolerance limits for frozen

tra fish *Pangasius hypophthalmus* fillets established by Vietnamese Ministry of Science and Technology [21] and the guidelines for fresh fish after production recommended by the Laboratory of Food Microbiology and Food Preservation (LFMFP) (Ghent, Belgium) [22] (Table 2).

Statistical analysis

The results of the microbial analysis of the water, fish and swab samples were expressed as log CFU/ml, log CFU/g and log CFU/100 cm^2, respectively. The results are reported as the mean value ± standard deviation. Differences in mean value during the three different visits and three independent sampling times were statistically assessed using one way analysis of variance (ANOVA) in SPSS version 20 (IBM Inc., Chicago, Ill., USA), when a Shapiro–Wilk test indicated that the means were normally distributed. The validity of the Shapiro–Wilk tests was assessed by means of various normality plots. If a Levene test confirmed heteroscedasticity, a Tamhane's *T2* test was chosen instead of Tukey's test. A non-parametric Kruskal–Wallis *H* type test was performed in case the data showed non-normality and comparison between paired means was performed using the Mann–Whitney *U* test. A non-parametric Spearman rank order correlation coefficient (r) was calculated for cross-correlations between the microbial counts investigated with a two-tailed test ($\alpha = 0.05$).

Table 2 Microbiological criteria

Parameters	Fresh fish in Belgian food industry[a]		Frozen tra fish (*Pangasius hypophthalmus*) fillet[b] Tolerance (log CFU/g)
	Goal (log CFU/g)	Tolerance (log CFU/g)	
Total aerobic psychotrophic count (TPC)	5.0	6.0	6.0
E. coli	2.0	3.0	2.0
Staphylococcus aureus	2.0	3.0	2.0
Vibrio cholerae	Absence in 25 g	Absence in 25 g	Absence in 25 g
Listeria monocytogenes	Absence in 25 g	Absence in 25 g	×
Salmonella spp.	×	×	Absence in 25 g

× no mention in the criteria

[a] for fresh fish developed by the Laboratory of Food Microbiology and Food Preservation (Ghent University), indicating 'Goal' and 'Tolerance' values (log CFU/g) [22]

[b] for frozen tra fish (*Pangasius hypophthalmus*) fillet established by Vietnamese Science & Technology Ministry [21]

Results

Dynamics of microbial quality and safety of *Pangasius* fillets during processing

A total of 144 samples were analyzed to establish the microbial quality of *Pangasius* fillets, water and food contact surfaces during processing. The variability of total aerobic psychrotrophic counts, coliforms, *E. coli* and *S. aureus* counts during the three different visits and three independent sampling times are shown in Fig. 2a–d, respectively. The total aerobic psychrotrophic counts, *E. coli* and *S. aureus* counts did not differ significantly ($p > 0.05$) between the three visits. However, the counts of coliforms increased significantly ($p = 0.013$) upon subsequent visits. More specifically, the counts of coliforms on the *Pangasius* fillet samples differed significantly between visits 1 and 2 ($p = 0.008$) and visits 1 and 3 ($p = 0.009$) (Fig. 2b). A strong correlation was observed between the counts of *E. coli* and coliforms ($r^2 = 0.747$, $p = 0.000$) (Fig. 3). The correlation among the other microbial parameters was not as strong as *E. coli* and coliforms. More specifically, correlation coefficients of 0.434, 0.522 and 0.211 were obtained between total aerobic psychrotrophic counts and counts of *E. coli* ($p = 0.000$), coliforms ($p = 0.000$), and *S. aureus* ($p = 0.045$), respectively.

The results of the microbial quality of the *Pangasius* fish at different processing locations can be seen in Fig. 4. It is clear that total aerobic psychrotrophic counts increased from 4.9 ± 0.8 on the raw materials to 6.0 ± 0.4 log CFU/g on the fillets sampled at the trimming step, after which they remained stable until the washing step. From the washing step onwards, the total aerobic psychrotrophic counts decreased slightly until they were 5.3 ± 0.4 log CFU/g on the fillet samples collected after packaging, the final processing step. Evolution of the coliform and *E. coli* counts during processing followed the trend observed for the total aerobic counts. Both coliforms and *E. coli* also increased from the raw materials (2.2 ± 0.6 and 1.1 ± 0.3 log CFU/g, respectively) to 3.9 ± 0.6 and 2.5 ± 0.4 log CFU/g, respectively, on the fillets sampled at the trimming step. Thereafter, the counts reduced to 3.5 ± 0.5 and 1.4 ± 0.5 log CFU/g, respectively, after packaging. As can be seen in Fig. 4, evolution of the counts of *S. aureus* did not follow the trend described above for the total psychrotrophic, coliform and *E. coli* counts. *S. aureus* occurred at low levels on samples of the raw material (1.4 ± 0.5 log CFU/g) and on the samples collected at the filleting, trimming, and washing steps. Thereafter, the *S. aureus* counts increased gradually from the washing step (SL 11) onwards to 2.3 ± 0.6 log CFU/g on the final products. The presence of pathogenic bacteria such as *L. monocytogenes, Salmonella* spp. and *V. cholerae* on the *Pangasius* fish was

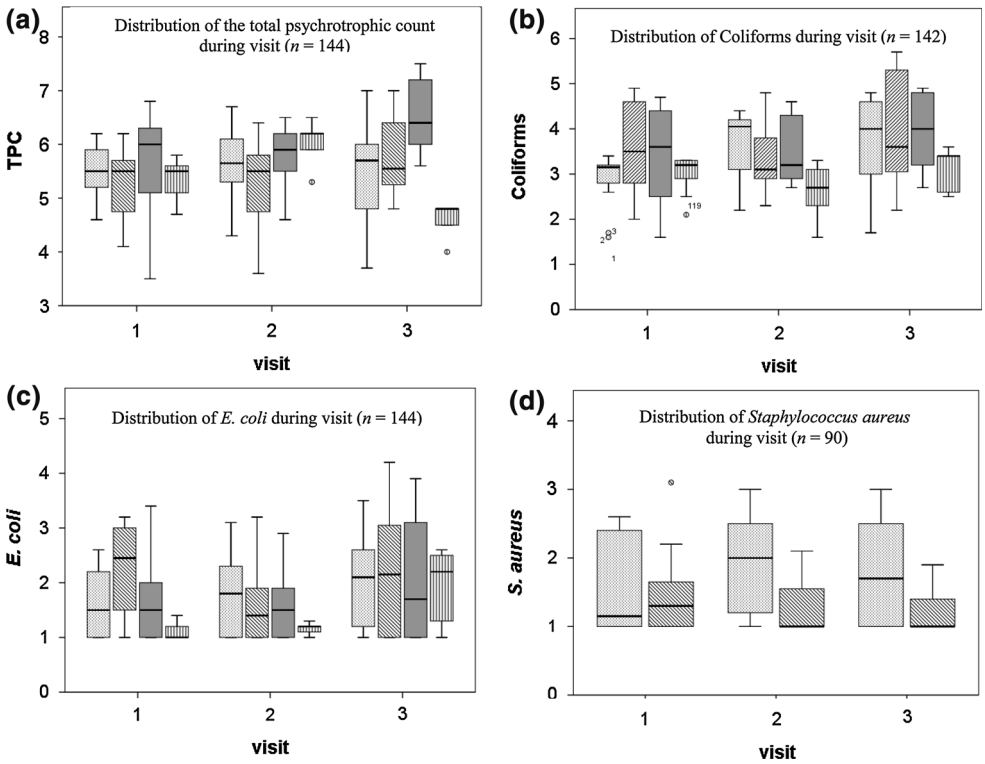

Fig. 2 The variability of total psychrotrophic counts (**a**), coliforms (**b**), *E. coli* (**c**), and *S. aureus* (**d**) between visits. *X* axis: visit 1 (ca. 8 a.m.), visit 2 (ca. 12 a.m.) and visit 3 (ca. 2 p.m.). *Y* axis: log CFU/g (fish), log CFU/100 cm^2 (hands and surfaces) and log CFU/ml (water). ▨ fish ▧ hands ■ surfaces ▥ water

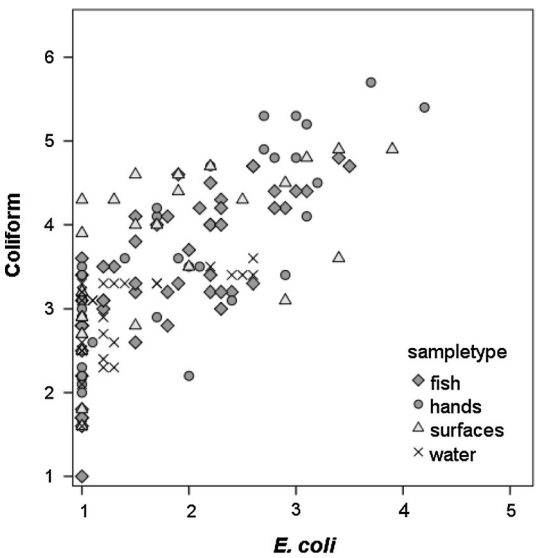

Fig. 3 Correlation of coliforms and *E. coli*. Fish (log CFU/g), hands and surfaces (log CFU/100 cm^2) and water (log CFU/ml)

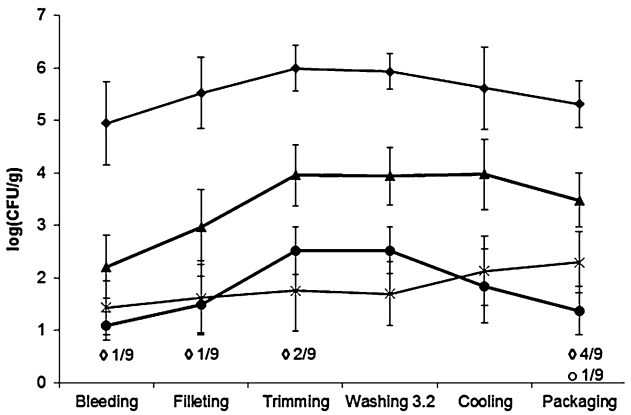

Fig. 4 Microbiological profile of fish samples during processing. ◆ TPC, ● *E. coli*, ▲ Coliforms, x *S. aureus*, ◇ *V. cholerae*, ○ *L. monocytogenes*

also investigated in this study (Table 1; Fig. 4). *Salmonella* spp. were absent in all fish samples investigated (Supplementary Table). *L. monocytogenes* was isolated from only one sample of the final product (a frozen *Pangasius* fillet), whilst *V. cholerae* was sporadically isolated from *Pangasius* samples at different processing steps: bleeding (1/9 samples), filleting (1/9), trimming (2/9) and packaging (4/9) (Fig. 4).

For the environmental samples, total aerobic psychrotrophic counts on the food contact surfaces ranged from 4.6 to 7.5, 5.5 to 7.4 and 3.5 to 6.6 log CFU/100 cm^2 at the filleting, trimming and packaging steps, respectively. Those on the hands/gloves ranged from 4.2 to 7.0, 5.1 to 6.6 and 4.1 to 6.2 log CFU/100 cm^2 at the filleting, trimming and packaging steps, respectively. On the hands/gloves, *E. coli* was found at high counts at the filleting and trimming steps, with the counts ranging from 1.7 to 4.2 and 1.7 to 3.7 log CFU/100 cm^2, respectively. On the food contact surfaces (tables, knives etc.), the counts of *E. coli* were <1–3.4 log CFU/100 cm^2. Low levels (close

to the limit of detection) of *E. coli* were found both on the hands/gloves (<1–1.1 log CFU/100 cm^2) and the food contact surfaces (<1 log CFU/100 cm^2) at the packaging step. *S. aureus* counts on the hands/gloves were <1–2.1, <1–1.7 and <1–1.9 log CFU/100 cm^2 at the filleting, trimming and packaging steps, respectively. *V. cholerae* was also detected on the hands/gloves of personnel at the filleting (2/6), cooling (2/6) and packaging (2/6) steps. *L. monocytogenes* and *Salmonella* spp. were not detected on the hands/gloves and food contact surfaces (Supplementary Table).

The microbial counts were high in the water sampled at the washing steps. The total aerobic psychrotrophic counts in the water samples ranged from 4.4 to 5.9, 4.0 to 6.4 and 4.5 to 6.5 log CFU/ml at the bleeding, washing 3.1 and washing 3.2 steps, respectively. Counts of the coliforms were 1.6–3.3, 2.3–3.6 and 3.2–4.7 log CFU/ml at the bleeding, washing 3.1 and washing 3.2 steps, respectively. *E. coli* was detected at low numbers in the water samples. As an example, the *E. coli* counts ranged from below the limit of detection (<1) to 1.7 log CFU/ml in water from the bleeding bath. *L. monocytogenes* and *Salmonella* spp. were absent in all the water samples collected while only one sample of water collected from washing step 3.1 was contaminated with *V. cholerae*.

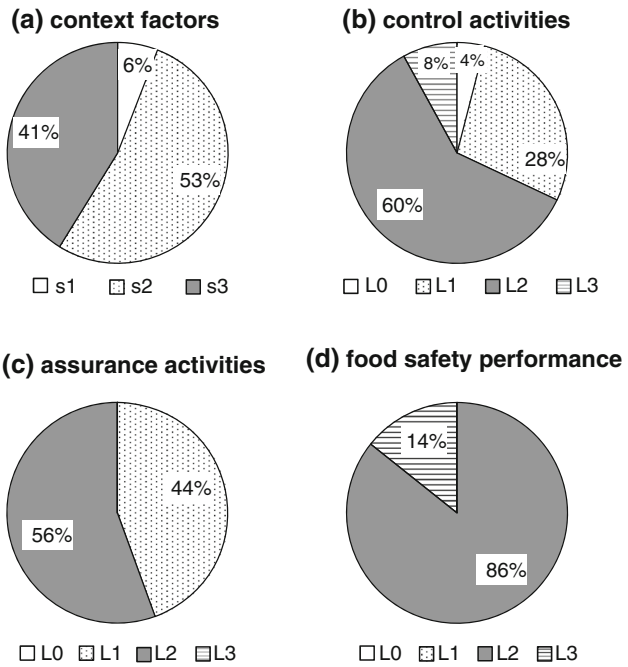

Fig. 5 Percentage of answers in various situations or levels in the self-assessment questionnaire on the food safety management system in place in the company for: context factors (**a**), control activities (**b**), assurance activities (**c**), and food safety performance (**d**). *s1* low vulnerability situation, *s2* moderate vulnerability situation, *s3* high vulnerability situation, *L0* absent, *L1* basic level, *L2* average level, *L3* advanced/tailored level

Results of self-assessment questionnaire

Figure 5a–d show the collated responses of the context factors, control activities, assurance activities, and food safety output, respectively. For the context factors, situation 2 (potential vulnerability) and 3 (high vulnerability) amounted to 53 and 41 % of the responses, respectively. Up to 60 % of the control activities correspond to level 2, indicating that they are done based on guideline information but not further tailored to the specific situation of the company we evaluated. Only 8 % of the control indicators were on level 3 and adapted to the specific situation of the company. The assurance activities were on level 1 (historical knowledge) with 44 % of the responses and level 2 (restricted level) with 56 % of the responses. Eighty-six percent of the QA staff evaluated their performance of food safety output as level 2 (standard follow-up) whilst 14 % indicated level 3 (comprehensive system evaluation).

Discussion

The MAS results provided insight into the actual microbial safety and quality of *Pangasius* fish processed in Vietnam. The assumption behind this study is that the FSMS of the company performs at an advanced level, meaning that the microbial counts on *Pangasius* fish during processing should be lower than the tolerance limits according to the guidelines for fish products after production [21, 22] (Table 2). The total aerobic psychrotrophic counts of the final products ranged between 4.6 and 5.9 log CFU/g. Although these counts did not exceed the tolerance limits, most of them exceeded the goal limit of 5 log CFU/g. The total aerobic counts of the final products in this study were greater than those observed by Noseda et al. [10] on products from a large-scale Vietnamese company with a daily production capacity of 200 tons.

The hygiene indicators, *E. coli* and coliforms, varied widely. As an example, counts of *E. coli* observed in the fish varied between <1 and 3.5 log CFU/g, whilst those in environmental samples varied between <1 and 4.2 log CFU/100 cm^2. These results were not consistent with those of the large-scale plant evaluated by Noseda et al. [10], where sporadic and low counts of *E. coli* were observed in the fish (<1–1.3 log CFU/g) and environmental samples (<1–2.1 log CFU/100 cm^2). In this study, the coliform counts on the fish sampled significantly increased on subsequent visits (Fig. 2b). The coliform counts were ca. 1 log CFU/g higher than those of *E. coli* (Figs. 3, 4) and a significant positive correlation occurred between these two microbial parameters ($p = 0.000$, $r^2 = 0.747$, Fig. 3). The

correlation between these two microbial parameters supports the idea that coliform counts could be used to predict the counts of *E. coli* [23]. However, Leclercq et al. [24] recommended the replacement of coliform analyses by *E. coli* enumeration as a means of estimating the sanitary quality of food. In addition, *E. coli* enumeration would likely give useful information as a quality indicator of fishery products, particularly the quality of *Pangasius* products [21, 22].

Most coliforms are present in large numbers in diverse natural environments, including the intestinal flora of humans and other warm-blooded animals, and are therefore harbored in fecal waste or freshwater bottom sediments or sands [25]. *E. coli* is the most common coliform in the intestinal flora of warm-blooded animals and is thought to be principally associated with fecal contamination [26]. Some previous studies have reported that Enterobacteriaceae (including *E. coli*) originate from the intestines of tropical freshwater fish, e.g. *Pangasius* [27], and possible routes of transmission occur from the filleting step onwards [9, 10]. Moreover, the *E. coli* and coliform counts on the *Pangasius* fillets sampled during processing in this study partly explain the high presumptive Enterobacteriaceae counts at the same small-scale factory determined previously by Tong Thi et al. [9]. Furthermore, cross contamination can occur when bacteria are transferred from food contact surfaces (i.e. hands, cutting boards and knives) to the food. High counts of both coliforms and *E. coli* were found on the hands and surface samples collected during processing. In contrast, low levels of *E. coli* contamination were found on the surfaces at the trimming step in the large-scale plant investigated by Noseda et al. [10]. The fact that high levels of indicator bacteria (coliforms and *E. coli*) were found on the hands and surfaces may also indicate that hygienic practices are insufficient in the company investigated in this study.

High levels of contamination were found in the water used to wash the fish. The fillets were washed manually by shaking a basket filled with 10 kg of fish fillets in tap water (ca. 100 l) in washing step 3.1 to remove dirt, fat and red muscle from the surface of the fillets. High total aerobic (4.0–6.4), *E. coli* (<1–2.6) and coliform (2.3–3.6 log CFU/ml) counts and the presence of *V. cholerae* (in 1 of 9 samples) were found in the water used at washing step 3.1. Therefore, there is a risk of cross contamination with pathogens from the washing water to the fish fillets. Therefore, the fillets might not be decontaminated during washing; this was shown as a no significant difference of total aerobic psychrotrophic counts on *Pangasius* fillets before and after washing [6.1 ± 0.6 and 6.0 ± 0.4 log CFU/g, respectively (data not shown)]. Moreover, to improve the microbial quality of fish after the trimming step, the fish fillets were washed in washing step 3.2 in water with 50 ppm chlorine. Unexpectedly, chlorinated water still showed high levels of bacteria e.g. 4.5–6.5, <1–2.6 and 2.3–3.5 log CFU/ml total psychrotrophic aerobes, *E. coli* and coliforms, respectively. During the visit, 50 ppm NaOCl was prepared for use at washing step 3.2 just before the shift started without adjustment of the pH and the chlorine concentration during processing. Therefore, the chlorine would have reacted with the organic matter in the water and fillets, resulting in a gradual loss of bactericidal activity [28]. In this study, the results also confirmed the findings of some previous studies which stated that chlorine is more effective for inactivation of pathogens in the wash water as opposed to its efficacy on pathogens on the product itself [29, 30]. For example, *V. cholerae* was absent in the chlorinated wash water used at step 3.2, whereas *V. cholerae* was detected (in 1/9 samples) in the tap water used at washing step 3.1. In addition, reduction of the bacterial load on the *Pangasius* fillets was not significant before and after washing with chlorinated water at trimming (SL 9) and washing 3.2 (SL 11) steps. This result suggests that the concentration of chlorine and the bacterial load in the washing water as a function of time should be further evaluated to increase the efficacy of the process.

As mentioned above, *V. cholerae* was found in the water at washing step 3.1. *V. cholerae* was also found for example in water at the bleeding step (1/9), on the hands of the workers at the filleting step (2/6) and on the *Pangasius* fish sampled at the filleting step (1/9). *V. cholerae* is a natural inhabitant of aquatic environments and has been isolated from the digestive tracts of fish [31]. This may explain why *V. cholerae* was detected on the fillets and the hands of the workers at the filleting step. In addition, *Vibrio* spp. have also been found in tropical water environments and fish are actually considered as reservoirs of *V. cholerae* [32]. However, in contrast to our findings, Noseda et al. [10] did not find any *V. cholerae* in the environment, water or *Pangasius* fillets from a large-scale *Pangasius* processing company in Vietnam.

On the other hand, *V. cholerae* was found in 4 out of 9 final packaged products. This might be a result of inadequate personal hygiene in the packaging area, as *V. cholerae* was found on the hands of food operators in the packaging area (2/6 operators). In addition, *S.aureus,* as an indicator of hand hygiene, was found on the hands of food operators (<1–1.9 log CFU/100 cm^2) at the packaging step. The *S. aureus* counts on five of the nine samples evaluated were greater than the limit of detection (>1 log CFU/100 cm^2), indicating that the hygiene practices were inadequate [22]. Moreover, *L. monocytogenes* was also found on one of the nine samples of the final product. *L. monocytogenes* is commonly found in water where fish are captured or cultivated, and in contaminated freshwater fish [33]. The transmission of *L. monocytogenes* into the final product has

been reported to occur from the fish raw materials and the processing environment [34, 35]. However, Hansen et al. [36] have reported that the incidence of *L. monocytogenes* is low in fish farms and the environment inside fish processing plants. In contrast, other studies emphasized that the processing environment was a route of transmission for *L. monocytogenes* into processed fish rather than directly from raw fish [34, 37]. As a result of the presence of *L. monocytogenes* and *V. cholerae* in the final *Pangasius* products of the company sampled in this study, it can be concluded that a potentially high food safety risk occurs, which should be addressed. The hygiene awareness and sanitation procedures in the production area should be revised, as cleaning and sanitation programs can have a great impact on reducing the presence of *L. monocytogenes* in the factory environment [37, 38].

The responses to the self-assessment questionnaire illustrated the actual status of the FSMS at the small-scale plant that was evaluated in this study (Fig. 5a–d). The assessment of products, processes, organization and environmental characteristics (Fig. 5a) indicated that the company was operating in a moderate to high vulnerability context. In comparison to the study conducted by Noseda et al. [10], this study showed that, with regards to the context situation, the product and process characteristics of the small-scale company were similar to those observed in a large-scale plant. Previous studies have also noted that the characteristics of the production process of *Pangasius* fillets by various companies in Vietnam are very similar [4]. In contrast, the organizational characteristics such as formalization and technological staff were high vulnerability (situation 3) whilst those of the large-scale company evaluated by Noseda et al. [10] were low vulnerability (situation 1). The company evaluated employs five people who work in the quality assurance department and all microbial analyses or safety controls are performed by external laboratories. Another weak point of the company evaluated in this study was not only the absence of activities in formal procedures but also the lack of formalized meetings e.g. meetings of the quality department. For the workforce and information systems, the company had a turnover of between 1 and 5 years, resulting in variability in the workforce. In addition, the information systems wherein information about safety processing, product, hazards, etc. is systematically recorded by the sampled company were less accessible to the staff. This characteristic of the information systems appeared to be independent of company size, as they were also reported by Noseda et al. [10] for a large-scale Vietnamese *Pangasius* company. The characteristics of the workforce and information systems indicated above provide more evidence of the high vulnerability in Vietnamese *Pangasius* processing companies in terms of organizational characteristics.

The control activities of the company sampled were less advanced since only 8 % of the response was at level 3. Most of the responses (60 %) were at level 2 which means that they are designed or conducted based on guidelines and best available equipment/materials in practice, but not tailored or tested for the company's specific situation, which can sometimes lead to an unstable performance. The sanitation program and personal hygiene requirements were less advanced because the guidance given by suppliers was implemented but no further improvements were made. Moreover, the monitoring system design was on level 1 since the standardized measuring equipment was neither tested for accuracy nor measured automatically e.g. using a portable thermometer. The calibration of the analytical equipment was on an ad hoc basis, moreover, the task and frequency of calibration programs was unclear and not documented. Regarding the plan and design for sampling (e.g. microorganisms), the company was designated level 1 due to its limitations in microbial expertise, lack of analysis facilities and strategies for improvement of the food safety and quality of processed products. The *Pangasius* products were sampled based on experience and in-house knowledge and analyzed by external laboratories without any checking by a third party. The actual performance of the analytical equipment used was level 0 as it was not calibrated by the company itself or by an external company. In contrast with these findings, the control activities in the FSMS of the large-scale company evaluated by Noseda et al. [10] were shown to be more advanced and adequate, accounting for 28 % of the responses for level 2 and 44 % of the responses for level 3. It can be derived that a less mature and tailored FSMS can be found in the small-scale plant we evaluated. The low level of control activities in the small-scale plant are correlated to the high levels of contamination found on the food contact surfaces, water and *Pangasius* samples. Therefore, the size of a company can indeed play a role in the further tailoring of the FSMS for certain activities such as sampling, microbial analyses, maintenance and calibration [39].

The core assurance activities were assessed at level 1 and 2 with 44 and 56 % of the responses, respectively. Specifically, the validation of preventive measures and intervention systems were level 1 as they were based on historical knowledge by the company itself. The validation of monitoring systems was also assigned level 1 since those validations of the company were based on historical and/or commonly available knowledge. The verification activities, documentation and record-keeping to support food assurance were level 2 because of the regular basis that these activities were performed and kept up-to-date in the documentation system (but not available online). In contrast to the results in the large-scale company [10], these activities were elaborated at higher levels (i.e. 33.3 % of the

responses at level 3 and 55.6 % at level 2). Often assurance activities lag behind control activities in food safety management systems: this has been demonstrated earlier in dairy companies in Japan [39] and in a large-scale European study [40]. Assurance activities are less clearly stated in food safety and hygiene legislation and demand extra effort from companies to implement; however, they are necessary to demonstrate the proper functioning of a FSMS [41].

In terms of external and internal food safety performance, the company was assessed with 86 % of the responses at level 2 (or six out of seven responses at moderate level of output) and 14 % at level 3 (or one out of seven responses at good level of output). The food safety output of this company often dealt with problems occurring with non-conformities, exceeding of microbial guidelines and complaints by customers (level 2). In addition, audit of the FSMS was performed yearly by one accredited third party. However, the product sampling was structured and conducted on the final product, raw materials and environmental samples using a fixed frequency sampling plan (level 3).

To conclude, although the general microbial quality of the final *Pangasius* products was acceptable from the point of view of the total aerobic psychrotrophic, *E. coli* and *S. aureus* counts, they were unacceptable with regards to some food safety parameters. The presence of hygiene indicators such as *E. coli* and *S. aureus* and of *V. cholerae* on the hands of the food operators during processing, particularly in the packaging area, reflects on the poor personal hygiene practices at the small-scale processing plant evaluated in this study. From the results of the MAS combined with the self-assessment questionnaire of the quality operators, it can be suggested that the core control activities (i.e. hand hygiene, cleaning and disinfection) should be greatly improved in order to develop adequate cleaning and sanitation procedures for equipment, personnel and the processing environment.

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