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Abstract

The two main variants (fermentation in aerobic and semi-aerobic conditions) of the traditional processing of Lanhouin were investigated with four processors in two different processing sites (Ativicondji and adovlocondji) in Grand Popo municipality with the objective to identify the impact of various unit operations on the microbial status of intermediate products at different steps of the processing, and the final product as well. The microbiological changes occurred at the main steps under observation were determined according to ISO methods described in the SOP. The follow up of processors revealed that, the two technologies consisted of same units operations except the fermentation step which was different according to the material used. The microbiological results showed that the total viable counts (TVC) of all the samples obtained with the two technologies were within the acceptable limits of < 5 Log cfu/g. The enterobacteriaceae, *Clostridium perfringens*, *Escherichia coli* and *Bacillus cereus* were found in few numbers (<1Log cfu/g) in the intermediate products and Lanhouin samples. For both type of fermentation, *Staphylococcus aureus* and CPS were detected in the majority (75 -100%) of samples at the beginning of the fermentation but their number decrease as fermentation progressed. Their loads ranged from 3.3± 0.1 to 3.7 ±0.5 Log cfu/g for the intermediate products (ripening fish) samples, and less than 1 to 1.8 Log cfu/g for Lanhouin samples obtained from both technologies. In return, no *Salmonella* and *Listeria monocytogenese* were found in any sample. The lack of good hygiene and good manufacturing practices were observed with the four processors monitored. The follow up had allowed to identify the critical control points along the processing diagram of Lanhouin and to suggest some corrective actions for the reengineering work.

1. Introduction

Lanhouin, a traditional fermented fish based condiment is mainly processed in the coastal areas of Benin; it is mostly used as taste enhancer and flavouring agent in many types of dishes (Anihouvi et al., 2005; Kindossi et al., 2012). However, its production is still artisanal; consequently the quality of the Lanhouin depends on the manner which the different unit operations of the process were conducted. Moreover, the most significant operations such as the salting, the ripening and the fermentation are not well defined, nor controlled whereas they determine the final quality of Lanhouin (Anihouvi et al., 2005; Kindossi et al., 2012). For reengineering purpose, it would be necessary to characterise the product on both microbiological and physico-chemical aspects during the processing to identify the role of each unit operation on the final quality of Lanhouin. Two mainly variants, aerobic fermentation and semi aerobic fermentation conditions were noted in the procedures employed for the processing of fresh fish into Lanhouin, but both lead apparently to the same end product (Anihouvi et al., 2005; Kindossi et al., 2012).

The processing sites are mostly located close to the beach; processing activities are carried out late in the evening or early in the morning and this was to avoid high temperature during the first stage of processing and to prevent contact of fish with flies. Activities are carried out mostly by illiterate women as the major executors (Anihouvi et al., 2005). The methods of processing were developed in homes and improvements were based on the observations of practitioners. There is little interest in knowing the role of micro-organisms and the chemical changes that occur in the product. What is recognized are changes in texture, colour, odour and taste. The current study aims to characterise Lanhouin processing diagram, to assess the microbiological quality and physic chemical of intermediate product and Lanhouin samples obtained from the two main technologies generally used to process Lanhouin, and to identify critical point of the process that have effect on the quality aspect of the intermediate product and end product.

2. Materials and methods

2.1 Follow up of processing

The follow up of Lanhouin processing was conducted in Grand Popo municipality (Ativicondji and adovlocondji sites) with four Lanhouin processors

2.2 Production variability

Four productions trials were done by considering the two popular technologies used by processors identified during the survey. The two variants of technology used for the processing were the fermentation in aerobic conditions with basket used as fermentation material (FA) and the fermentation in semi-aerobic conditions with plastic barrel or basket with cement layer, and plastic can used as fermentation materials (FSA). The type of fish used during the follow up of the two technologies is the fatty fish named king fish/Spanish mackerel supplied.

2.3 Diagram establishment

The parameters raised during the diagnosis were different orders. The operational variables, flows of matters, inputs and waste were taken into account to work out the diagram of manufacture. The qualitative description of the process was focused preferably on photographs during manufacture. The list of the equipment used by the operators with each steps were included in this description. Sampling was done on products at different steps to make analyses in laboratory.

The diagram was established according to the model annex 1, which is the example of the process of production of Lanhouin in Benin.

For each unit operation (OU), the necessary information have been noted:

- Principal operational variables:
    - Duration of unit operations
    - Temperatures recording (initial, final, ambient)
    - In the diagram, in column "Analysis-follow-up-sampling" is specified as well as simple measurement of Ti, Tf or T_{amb}, .
- The matter flow for the principal product: initial mass, final mass. These masses can be different for: (i) the process caused losses by exudation, (ii) waste was extracted (viscera, scale, etc…) or (iii) the fish received the addition of ingredients (salt, water, etc…). Moreover, samples were taken at the end of OU. The sampling did not enter in the calculation of the output of previous OU. To be capable to take into account only the variations related to OU and to make partial or total balance, the fish weights were expressed on a basis 100 initial throughout process.

Flows of input:

- Volumes of water (washing)
- The number of labour necessary to the realization of OU.

- Waste matter:
  - Volumes of waste water
  - Viscera and scales

The steps where sampling was done for microbiological analyses were PA1, PA2, PA3 and PA4 (Table 1).

2.4 Sampling variability during the follow up of production

The sampling was done on product obtained from step undergo biological, chemical and physical phenomenon. The selected steps were (annex 1: Diagram of production and sampling):

- Ripening: at the end of this step, the fresh fish is in partially deteriorated form; thus chemical and microbiological changes are expected. Chemical compound such as total volatile nitrogen (TVN) and biogenic amines which normally did not exist in the living tissues of fish are formed by autolysis and microbial actions. This step influence on the texture (soft) and on the aroma of the end product. (PA1: soft fish)

- Salting: the fish is salted with variable amount of salt (sodium chloride) to reduce microbial growth and that appeared during ripening. This step influences the colour (bright, shining) and the aroma of the end product. (PA2: salted soft fish)

- Fermentation: the fermentation of fish is a partial broken down phenomenon of complex organic molecules to simpler ones due to enzymes (autolysis) and micro-organisms present in the fish flesh. This is controlled by the addition of salt, thus the process is designed to produce a particular flavour and to preserve the end product as well (PA3: fermented fish)

- Drying: the salted and fermented fish is sun dried to reduce the moisture and stabilized the water activity. (PA4: Lanhouin)

In the same way, this work took into account the establishment of the matter balance during the production as well as the identification of the critical control points of the process, and other sources of danger or potential degradation of the quality of the end product. This consisted of the observation of fish and processing materials handling at all the steps of process and hygiene of the processing environment

The sampling steps and the microbiological parameters analysed are summarized in table 1.

Table 1: Sampling steps and microbiological criteria analysed

Sampling steps	PA1	PA2	PA3	PA4
Microbiological criteria				
Enumeration of microorganisms	x	x	x	x
Enterobacteriaceae	x	x	x	x
Escherichia coli	x	x	x	x
Bacillus cereus	x	x	x	x
Staphylococcus aureus and CPS	x	x	x	x
CN Staphylococcus	x	x	x	x
Lactic acid Bacteria	x	x	x	x
Yeast and Mould	x	x	x	x
Clostridium Perfringens		x	x	x
Salmonella spp	x	x	x	x
Listeria monocytogenese			x	x

PA1: soft fish; PA2: salted soft fish; PA3: fermented fish; PA4: Lanhouin: fermented and dried fish

3. Results and discussion

Description and variability of processing methods (with flow diagrams, equipments and promising technology)

Processing

Both technologies monitored include seven units operations which are: the first washing, the dressing (scaling and gutting), the second washing, the ripening, the third washing, the salting, the fermentation and the sun drying. These different steps are showed in figure 1. For the fermentation in aerobic conditions the basket was use as fermentation material while for the fermentation in semi-anaerobic conditions, a plastic can, a plastic barrel and a basket with cement layer were used as fermentation materials.

For processing of Lanhouin, fresh king fish (weighing 7.2 ± 0.92 for the first trial and 9.65 ± 0.21 kg for the second trial) was washed using well water (collected near the processing site) and gutted with knife to remove the viscera. The weight of viscera rejected varied between 0.25 ± 0.0 and 0.28 ± 0.11 kg (Table 2). Then the dressed fish was washed again with four (04) litres of water. The dressing step was followed by the ripening step which consists of living the fresh fish without any treatment. For the ripening, the dressed and washed fish was arranged in a plastic can or plastic of aluminium bowl, and covered with another plastic of aluminium bowl, and left for an average time of 10.5 hours at ambient temperature (30 ± 2°C). During this step, the fish was subjected to a process of tissue degradation under enzymes and microorganisms activities. After the 10.5 hours of ripening, the seemingly spoiled fish weights were 6.50 ± 0.21 for the first trial and 9.04 ± 0.45 kg for the second one. Dry salt (4.68±0.25 and 4.70±3.11 kg) was introduced into the slit of evisceration under the operculum, into the gills and was passed on all the fish body. After the salting, the salted fish weighing 11.08±0.11 in the case of aerobic fermentation trial and13.74±3.56 kg for semi-aerobic fermentation trial, (Table 2), was arranged in a basket (aerobic fermentation condition), a plastic can or plastic barrel or a basket with cement layer (semi aerobic fermentation); the rest of amount of salt used for curing was added and the fish was covered with old cement paper bag and old clothes, and allowed to ferment for nine (9) days at room temperature (30 ± 2 °C) before being removed (Figure 1, photo1-8). At the end of fermentation, the salted fermented fish weighed 6.70±0.21 and 9.05±0.07 kg for aerobic fermentation and semi-anaerobic fermentation respectively (Table 2). The salted and fermented fish was then rinsed (Photo 12) to reduce the excess of salt (1.95±0.07 and 2.38±1.11 kg for aerobic fermentation and semi-aerobic fermentation condition respectively) and was sun dried for average time of 8 hours. The weight of Lanhouin (dried salted fermented fish) was 3.89±0.05 and 5.33±0.39 kg for aerobic fermentation and semi-aerobic fermentation respectively. No significant difference was observed for the weight of Lanhouin from both technologies. The table 3 summarized the equipments used per processing steps.

Table 2: Weight variations during Lanhouin processing

Products	Weight (kg)	
	Aerobic fermentation	Semi aerobic fermentation
Fresh fish	7.20±0.92	9.65±0.21
Dressed Fish	6.95±0.92	9.38±0.32
Washed dressed Fish	6.90±0.92	9.34±0.31

Soft dressed fish	6.50±0.21	9.12±0.42
Washed soft Fish	6.40±0.35	9.04±0.45
Salted soft fish	11.08±0.11	13.74±3.56
Fermented fish	6.70±0.21	9.05±1.17
Rinsed fermented fish	4.75±0.14	6.67±0.07
Lanhouin	3.89±0.05	5.33±0.39

Table 3 : Utensils, etc used for Lanhouin processing

Processing steps	Utensils, etc
Washing	Plastic or aluminium bowl
Gutting	Knife
Ripening	Plastic can, or plastic bowl
Salting	Plastic or aluminium bowl
Fermentation	Basket with cement layer, plastic can, plastic barrel
Washing off salt	Bowl
Storage and packaging for sale	Basket, cement paper bag




Washed fresh fish (1) Dressing (2) Washed dressed fish (3) Ripening (4)




Soft fish (5) preparation of basket (6) Dry salting (beginning)(7) Dry salting (end)(8)




Aerobic fermentation (basket) (9) Semi aerobic fermentation (can) (10)




Fermented fish (11) Rinsing (12) sun drying (13) Lanhouin (14)

Figure 1: processing steps of Lanhouin

Microbiological results

The results of microbiological data recorded on the intermediate products and Lanhouin samples are summarized in tables 4 and 5. Before washing, a total viable count (TVC) loads of 5.36 ± 1.7 was obtained for fresh cassava fish, but after washing, the TVC loads enumerated for fresh fish cassava fish were 4.80 ± 0.7 Log cfu/g while TVC loads of 5.8 ± 0.2 and 5.5 ± 0.6 were recorded for the ripening fish (soft fish) during aerobic and semi-aerobic fermentations trials respectively. These levels of microbial loads could be considered as the initial loads at the beginning of the fermentation just after salting. The enterobacteriaceae, loads in the ripened fish ranged from 3.2 ± 0.4 to 3.6 ± 0.8 Log CFU/g whereas microbial loads ranging from 3.3±0.1 to 3.7±0.5 were recorded for *Staphylococcus aureus* and coagulase positive *Staphylococcus* (CPS) for the trials in semi-aerobic and aerobic conditions respectively. In return, *Escherichia coli*, *Bacillus cereus* and *Clostridium Perfringens* were present in few numbers (< 1 Log cfu/g) in the ripened fish while *Salmonella* and *Listeria monocytogenese* were absent.

Regarding the salted fish, their microbial loads decreased as fermentation progressed. The TVC loads of fish fermented in aerobic conditions decreased from 5.8 ± 0.2 to 4.5 ±0.6 Log cfu/g for nine days fermented fish samples and 4.7 ± 0.8 Log cfu/g for Lanhouin samples (Table 4). The same observation was made for fish samples fermented in semi-aerobic conditions; their TVC decreased from 5.5 ± 0.6 to 4.4 ±0.1 Log cfu/g for nine days fermented fish samples and 4.6 ± 0.6 Log cfu/g for Lanhouin samples (Table 5). These results showed that the fermented fish are slightly contaminated during step. Similarly, for both type of fermentation, *Staphylococcus aureus* and CPS loads decreased with fermentation time (from 3.7 ± 0.5 Log cfu/g to 2.0 ± 0.2 for samples fermented for 9 days and 1.8 Log cfu/g for Lanhouin samples for aerobic fermentation, and from 3.3 ± 0.1 to <1 Log cfu/g for samples fermented for 9 days and for Lanhouin as well). In contrast, coagulase positive *Staphylococcus* (CNS) number increased with fermentation time for both type of fermentation (Tables 4 and 5). The enterobacteriaceae loads were <1 Log cfu/g after 3 days of fermentation for both types of fermentation.

Both lactic acid bacteria (LAB) and yeast and moulds were present in few numbers in the ripened fish; but they disappeared as the fermentation progressed in the case of aerobic

fermentation while their number increased slightly with fermentation time in the case of semi-aerobic fermentation (Tables 4 and 5).

Based on these results, it appeared that the first the washing contributed to reduce the microbial loads of fresh fish while the salting contributed to increase the microbial loads at the beginning of the fermentation, but contributed also to control the microbial loads as the fermentation progressed. The gradual decrease observed during the fermentation trials suggested that the less halophilic micro organisms were eliminated, giving way to the more halophilic organisms already present in the fish and the added salt. The inhibition is due to the bactericidal and bacteriostatic property of salt, and the dehydration or osmotic action of salt, resulting in a lower water activity of the fermenting fish samples, thus making it impossible for certain microorganisms to survive in the environment. In Addition, the quality of the salt used to cure the fish is determinant; in this respect, the presence of *Staphylococcus aureus* and CPS in the fermenting fish and Lanhouin could be attributed to the quality of salt used (Figure 1, photo 8). Drying conditions also had a negative influence on the quality of the end-product. It was observed that the TVC of fermenting fish samples slightly increased after drying.

Table 4: Microbiological status of intermediate products and Lanhouin obtained from aerobic fermentation process

Microoganisms	SOP number	soft fish	salted fermented fish (3^{th} day)	salted fermented fish (6^{th} day)	salted fermented fish (9^{th} day)	Lanhouin
Enumeration of microorganisms	Micro-01, ISO 4833	5.8±0.2	5.3 ± 0.3	4.9±0.1	4.5±0.6	4.7±0.8
Enterobacteriaceae	Micro-02, ISO 21528-2	3.2±0.4	<1	<1	<1	<1
Escherichia coli	Micro-03,ISO 16649-2	<1	<1	<1	<1	<1
Bacillus cereus	Micro-04, ISO 7932	<1	<1	<	<1	<1
Staphylococcus aureus and CPS	Micro-05, ISO 6888-1	3.7±0.5	3.4±1.3	ND	2.0±0.2	1.8.0±0.0
CN Staphylococcus	Micro-05, ISO 6888-1	2.5 ±0.1	3.9±0.2	ND	4.0±0.1	3.9±0.1
Lactic acid Bacteria	Micro-10, M-METH-MO-13	1.7±0.1	<1	ND	1.1±0.0	<1
Yeast and Mould	Micro-09, ISO 7954	2.3±0.8	<1	<1	<1	<1
Clostridium Perfringens	Micro-08, ISO 7937	1.4±0.0	ND	ND	<1	<1
Salmonella (search in 25 g sample)	Micro-07, ISO 6579:	A	ND	ND	A	A
Listeria monocytogene (search in 25 g sample)	Micro-06, ISO 11290 1/A1:2004	A	ND	ND	A	A

A: absence in 25 g; ND: not determined

Table 5: Microbiological status of intermediate products and Lanhouin obtained from semi aerobic fermentation process

Microoganisms	**SOP number**	soft fish	salted fermented fish (3th day)	salted fermented fish (6th day)	salted fermented fish (9th day)	Lanhouin
Enumeration of microorganisms	Micro-01, ISO 4833	5.5±0.6	5.1±0.2	4.6±0.2	4.4.0±0.1	4.6±0.6
Enterobacteriaceae	Micro-02, ISO 21528-2	3.6±0.8	<1	<1	1.4±0.0	1.3±0.0
Escherichia coli	Micro-03,ISO 16649-2	<1	<1	<1	<1	<1
Bacillus cereus	Micro-04, ISO 7932	<1	<1	<1	<1	<1
Staphylococcus aureus and CPS	Micro-05, ISO 6888-1	3.3±0.1	3.1±0.1	2.2±0.0	<1	<1
CN Staphylococcus	Micro-05, ISO 6888-1	2.8±0.1	3.9±0.1	4.1±0.0	3.8±0.0	3.9±0.3
Lactic acid Bacteria	Micro-10, M-METH-MO-13	1.5±0.1	3.1±0.1	2.7±0.2	2.7±0.0	2.9±0.1
Yeast and Mould	Micro-09, ISO 7954	2.0±0.5	ND	ND	1.4±0.0	1.2±0.0
Clostridium Perfringens	Micro-08, ISO 7937	<1	<1	<1	<1	<1
Salmonella (search in 25 g sample)	Micro-07, ISO 6579:	Absent	ND	ND	Absent	Absent
Listeria monocytogène (search in 25 g sample)	Micro-06, ISO 11290-1/A1:2004	Absent	ND	ND	Absent	Absent

A: absence in 25 g ; ND: not determined

Table 6: Identified of critical control points and corrective actions

Designation of the "hazard", the problems (note from 6 to 9)	**Operation (step) concerned**	**Possible cause *(see listing under the table)***	**Corrective actions suggested (reengineering/implantation of one OU)**	**Feasibility ***
6	Dressing (evisceration, scaling)	Microbial cross contamination of fish by viscera; by processing materials	Make sensitive the processor to put viscera in a vat envisaged so that it does not be in contact of dressed fish dressed or no dressed fish; training in fish handling and Good hygiene practices including materials hygiene	Awareness
	Washing1	Microbial cross contamination of water to fish	Use of potable water, evaluate microbiological status of washed dressed fish; Good hygiene practices including materials hygiene	Potable water should be used wherever necessary to avoid cross

				contamination.
	Ripening	Proliferation of microorganisms and biogenic amines production in the fish	Combination of ripening and salting to limit both microbial proliferation and biogenic amines production. The salting will be done by immersion in brine	Ripening by immersing the gutted fish in a solution of citric acid or ascorbic acid
	Washing 2	raw materials (drinking water)	Use of potable water, evaluate microbiological status of washed dressed fish	Potable water should be used wherever necessary to avoid cross contamination.
	Salting	Ingredients (salt)	Do not use any salt provide from source or doubtful quality	
	Fermentation	Surfaces, utensils, equipment, should be thoroughly cleaned and where necessary disinfected after raw food (fish), has been handled or processed.	Fermentation in wood box or plastic; good hygiene practices including materials hygiene Development of starter culture for the controlled fermentation (biopreservation)	
	Washing 3	Microbial contamination by flies and environment	Good hygiene practices including materials hygiene; use of repellent agent.	Addition of citric acid or ascorbic acid to the water before washing
	Drying	sun drying (favour the contamination of product, since it is done in very unhygienic conditions)	develop drying equipment	
	Packaging	Protection of end product	Develop of packaging	Presentation of new types of Lanhouin (Lanhouin in form of dried fillet or in form of powder)

The standard fields of possible causes are indicated below:

- ***Raw product****: raw materials, ingredients, storage, quality*
- ***Material****: machines, tools, equipment, capacity, age, a number, maintenance, quality*
- ***Labour****: formation/competence, absenteeism, motivation, hygiene of the personnel, painfulness of the tasks, ergonomics…*
- ***Medium(environment)****: physical environment, utilities (water, energy), lighting, noise, installation, temperature, climate*
- ***Methods****: instructions, handbooks, procedures, steps procedures*
- ***Measurements****: control*

- ***Management****: organisation*
- ***Finished product****: outgoing element*

Matter balance, yield and processing time

The follow-up of the mass variations was used to determine the yield in wet weight basis and the processing time of each unit operation (Table 2 and annex 2 matter balance for aerobic and semi-aerobic fermentations).

Three unit operations ripening, fermentation and drying take more time, with average of 10.5 hours, 9 days, 10 hours respectively. Significant difference ($p < 0.05$) was observed for the processing time between the fermentation in semi- aerobic conditions (233 hours ≈ 9 days 16 hours) and fermentation in f aerobic conditions (240 hours ≈ 10 days) (Figure 2). This difference was mainly due to the ripening duration (10.56 hours) realized during semi aerobic fermentation which was lower than the ripening duration (13.78 hours) obtained for aerobic fermentation. The yield of the unit operations (OU) varied very slightly from one technology to another that indicates a comparable loss according to technology. Significant difference was observed for global yield (55.6 ± 2.0%) obtained from semi aerobic fermentation compared to that (54.0 ± 6.1) obtained for aerobic fermentation. Matter loss was observed mainly during fermentation (matter loss 49.1% for semi-aerobic fermentation and 61.7% for aerobic fermentation) and sun drying (23.9% for semi-aerobic fermentation and 12.2% for aerobic fermentation). The weight losses during the fermentation and sun drying steps were due to dehydration effect of salt and water evaporation from end product during sun drying respectively.

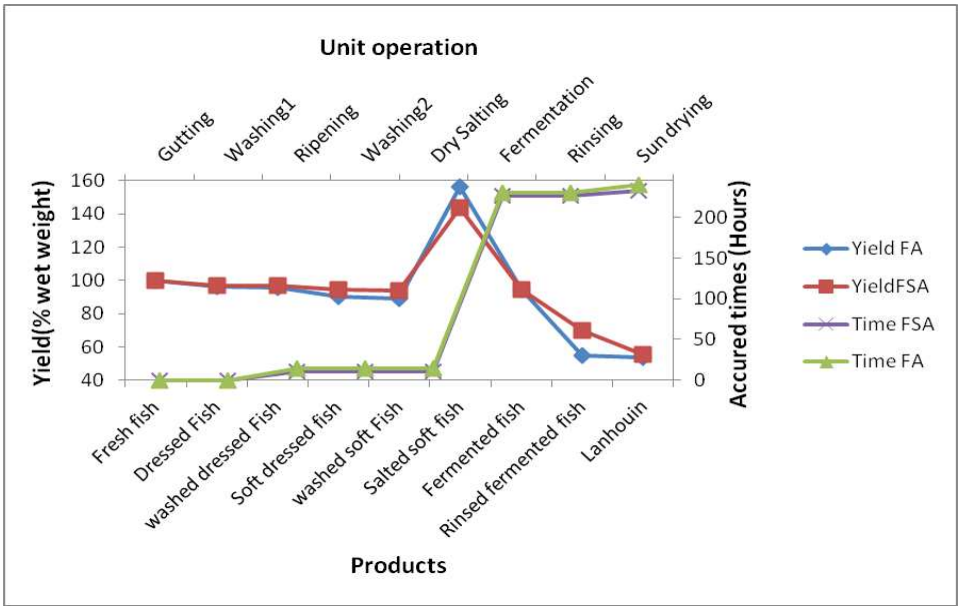
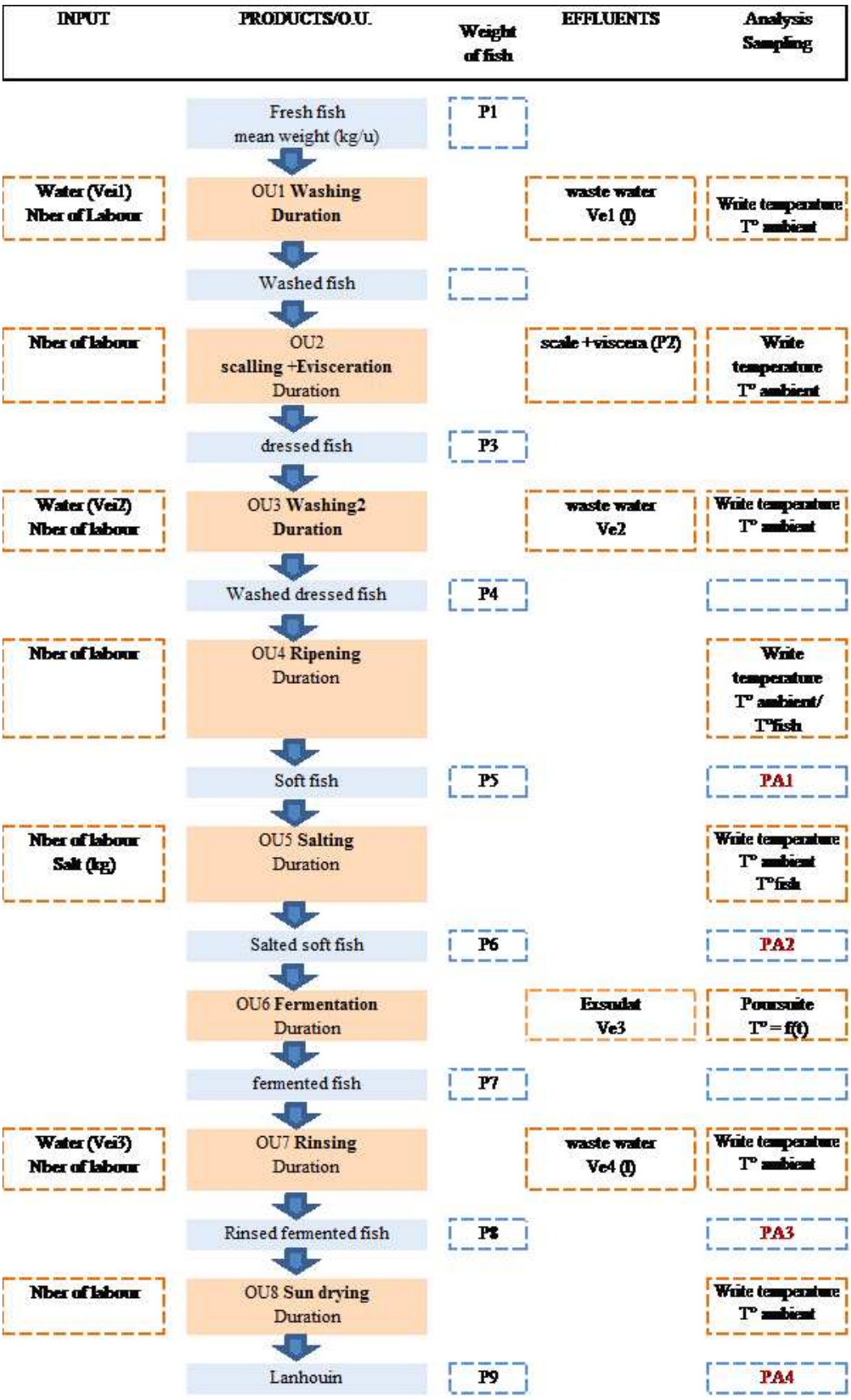


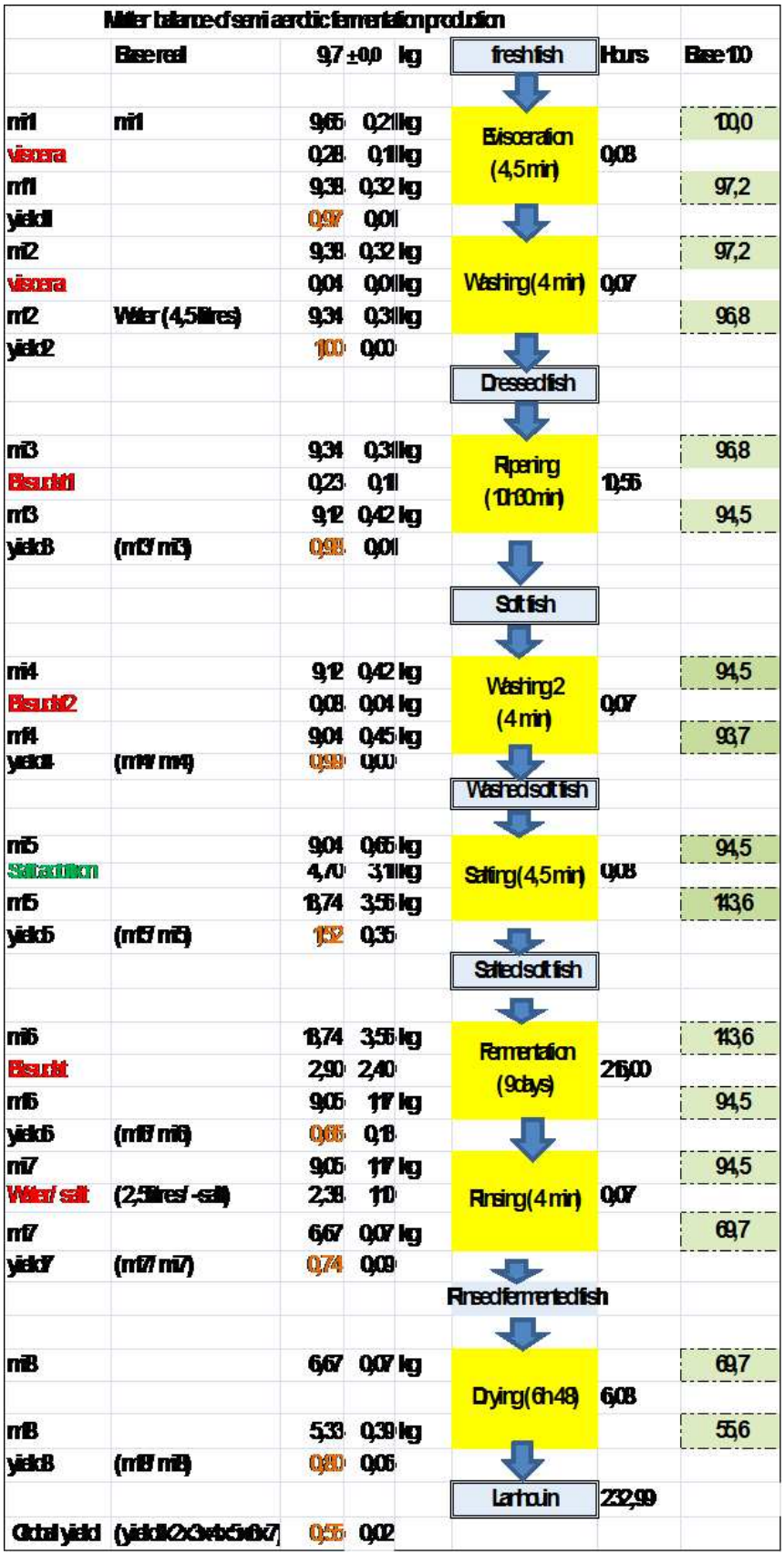
Figure 2: Yield of intermediate products, yield Lanhouin and processing times

FA: aerobic fermentation, FSA: semi-aerobic-fermentation

Annex 1



Annex2 matter balance of aerobic fermentation condition



Annex 3: matter balance of semi aerobic fermentation condition

Mass balance of aerobic fermentation production								
	Base real	7,2	±0.0	kg	Fresh fish	Hours	Base 100
m1	m1	7,20	0,92	kg	Evisceration (2,5 min)	0,042	100,0
Viscera		0,25	0,00	kg			
m1		6,95	0,92	kg			96,5
Yield1		0,97	0,00				
m2		6,95	0,92	kg	Washing (3,5 min)	0,058	96,5
Viscares		0,05	0,00	kg			
m2	Water (4,5 litres)	6,90	0,92	kg			95,8
Yield2		0,99	0,00				
					Dressed fish		
m3		6,90	0,28	kg	Ripening (18 h 45 min)	18,78	95,8
Discard1		0,40	0,00				
m3		6,50	0,28	kg			90,3
Yield3	(m3/ m2)	0,94	0,00				
					Salt fish		
m4		6,50	0,35	kg	Washing 2 (3,5 min)	0,058	90,3
Discard2		0,10	0,00	kg			
m4		6,40	0,35	kg			88,9
Yield4	(m4/ m4)	0,98	0,00				
					Washed salt fish		
m5		6,40	0,64	kg	Salting (6 min)	0,1	90,3
Salt addition		4,68	0,25	kg			
m5		11,08	0,11	kg			156,2
Yield5	(m5/ m5)	1,73	0,10				
					Salted salt fish		
m6		11,08	0,11	kg	Fermentation (9 days)	216	156,2
Discard		3,48	0,11				
m6		6,70	0,21	kg			94,5
Yield6	(m6/ m6)	0,60	0,01				
m7		6,70	0,21	kg	Rinsing (3,5 min)	0,05	94,5
Water/ salt	(2,5 litres/ -salt)	1,95	0,07				
m7		4,75	0,11	kg			67,0
Yield7	(m7/ m7)	0,71	0,00				
					Rinsed fermented fish		
m8		4,75	0,11	kg	Sun drying (10 h)	10	67,0
m8		3,89	0,05	kg			54,8
Yield8	(m8/ m8)	0,82	0,03				
					Lanhouin	240,1	
Global yield	(Yield1x2x3x4x5x6)	0,51	0,05				