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Verification of a new processing method for treatment of category 2 material of fish origin – Full Scale Trials

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Summary/recommendation:

The Norwegian Seafood Federation (FHL) has applied for approval of a new alternative method for processing of category 2 animal by-products (ABP) of fish origin. The processing method is characterized by fish raw material that is grinded before mixing with formic acid at pH ≤4 and stored for ≥24 hours before heat treatment of the silage with a particle size ≤10 mm at a temperature ≥85 °C for ≥25 minutes.

The method was assessed by EFSA (BIOHAZ-panel) who concluded that the risk related to pathogens present in fish ABP's from aquaculture would be adequately reduced by the proposed process if the requirements of the HACCP-plan could be achieved in a full scale plant. The production trials verified the feasibility of the HACCP-plan under real processing conditions in a full scale plant.

Another purpose of the production trials was to demonstrate that relevant pathogens are inactivated by the proposed process. For each of 20 batches produced, 5 samples of silage and 5 of end product were analyzed for *Salmonella* and *Enterobacteriaceae*. All results were negative, confirming earlier lab scale experiments showing that the formic acid treatment alone inactivate those bacteria. One composite sample of silage and one of end product for each batch were analyzed for *C.perfringens*. All results were negative, confirming a low prevalence of *C.perfringens* in fish silages, as also demonstrated in earlier surveys. Anaerobic sulfite-reducing bacteria were analyzed in the same composite samples. The concentration in silage ranged from 1.100 to 130.000 per gram, while heat treatment provided on average more than 3 log reductions. This reduction rate is comparable to that found in earlier lab scale inactivation experiments with *C.perfringens* and *C.sporogenes* spores.

All batches of end product met the microbiological requirements used to assess inactivation effect of new processing methods according to method 7 in Regulation (EC) 1774/2002. Based on this, it is concluded that the risk related to pathogens present in fish ABP from aquaculture would be adequately reduced by the proposed process.

Summary/recommendation in Norwegian:

Fiskeri og Havbruksnæringens Landsforening (FHL) har søkt om godkjenning av en ny alternativ metode for prosessering av kategori 2 bi-produkter fra fisk. Metoden består av oppmaling og maursyrebehandling av fisken ved pH ≤4 i ≥24 timer før varmebehandling av fiskeensilasje med partikkelstørrelse ≤10 mm ved ≥85 °C i ≥25 minutter. Metoden ble vurdert av EFSA (BIOHAZ-panel) som konkluderte at risiko knyttet til patogener i biprodukter fra akvakultur ville bli tilstrekkelig redusert hvis kravene i HACCP-planen lot seg gjennomføre i fullskala.

Rapporten beskriver en serie produksjonsforsøk som viste at HACCP-planen var gjennomførbar under realistiske forhold i et fullskala fabrikkanlegg og at relevante patogener ble inaktivert gjennom prosessen.

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1 Introduction

Norwegian Seafood Federation (FHL) has applied for approval of a new alternative method for processing of category 2 animal by-products (ABP) of fish origin, according to Regulation (EC) 1774/2002.

The processing method is characterized by fish raw material that is grinded before mixing with formic acid at pH ≤4 and stored for ≥24 hours, before heat treatment of the silage with a particle size ≤10 mm at a temperature of ≥85 °C for ≥25 minutes.

The end product of the new process is heat-treated fish silage. It may be placed on the marked as such, i.e. as feed ingredient for fur animals, as substrate for biogas or bioenergy production, for technical use or as a fertilizer, etc. It may also be placed on the marked for further processing and separation of the oil from the protein/water phase. The fish oil may be used as fuel, for technical use, fertilizer or feed ingredient of fur animals, etc. The protein containing water phase may be evaporated to decrease its water content, resulting in a protein concentrate. The protein phase may be used as feed ingredient for fur animals etc. or as a fertilizer.

The method was assessed by EFSA (BIOHAZ-panel) for the inactivation of relevant biological hazards. The Panel concluded that, based on the results of laboratory experiments, the risk related to pathogens present in fish ABP's from aquaculture would be adequately reduced by the proposed process if the requirements of the HACCP-plan are achieved. It was further recommended to verify the feasibility of the HACCP-plan in a full scale plant, where records of the main parameters (time, pH, temperature) should be assessed for a certain period under real operating conditions.

The purpose of the verification trials was to demonstrate that the requirements of the HACCP-plan can be achieved during full scale production, that relevant pathogens are inactivated by the new processing method and that the end products are safe.

The trials were conducted February 12. - 20. 2013. The project leader attended the first day of production, where operating practices were reviewed and agreed.

2 Materials and methods

2.1 Project organization

The project was funded by a grant from The Norwegian Seafood Research Fund (FHF) and conducted by Nofima AS in cooperation with the project team;

Gunn Harriet Knutsen	Advisor Health & Quality, Norwegian Seafood Federation (FHL)
Jørgen Seliussen	Quality Manager, Hordafor AS
Oddvar Aftret	Head of Production, Scanbio AS
Halvor Nygaard	Project leader, Nofima AS

The project was supervised by a reference group who reviewed the project plan and the draft report;

Berit Johnsen	Head of Market & Quality, Scanbio AS
Gunnar Grønvoll	Head of Research & Devolepment, Akvaren
Kjell Wergeland	Senior Advisor, Norwegian Food Safety Authority

2.2 Production facilities and process description

The verification trials were conducted at Scanbio K2 AS, February 12th - 20th 2013. Scanbio K2 is located in Lysøsund and is one of Scanbio's three processing plants for fish by-products.
Scanbio K2 AS is approved by the Norwegian Food Safety Authority as a processing plant for category 2 materials of fish origin according to Regulation (EC) No 1774/2002. The material is treated by method 1 (pressure sterilization) as currently required by 1774/2002 and by 1069/2009 and 142/2011 accordingly.

In order to be able to run the new alternative method, some of the processes had to be modified. The modifications were:

- New pipes and valves to be able to run alternative process trial
- New temperature transmitters to be able to log temperature
- Modification of control system to be able to control the modified process and run 85 ºC for 25 minutes, and to log temperature in the heat treatment tank
- Resetting of control system in-between the days of trial production

After heat treatment with the new alternative method, the whole batch had to be reprocessed with method 1 according to current legislation, before placing on the marked.

Fish silage production

At the place of origin, the aquaculture production sites, category 2 fish by-products are on a daily basis collected, grinded and mixed with formic acid to pH ≤4 before it is filled into storage tanks. The fish silage is normally stored at the place of origin for more than 24 hours before transport to the processing plant. Immediate grinding and acidification of category 2 fish by-products at the aquaculture production site is mandatory according to the Norwegian regulations and the purpose is to prevent spreading of fish diseases.

Transport of fish silage

The fish silage was transported from the aquaculture production site in closed tanks by vessels especially designed for this purpose. The transport and the vessel management system are according

to the hygiene requirements of Regulation (EC) No 1774/2002 Annex II. This includes the use of commercial documents and keeping of records both by the consignor and the transporter.

Processing

The fish silage was processed in a closed production line with tanks and pipelines. Received silage was pumped into closed storage tanks and further through a heat exchanger and a sieve before transfer to a 10 m^3 unagitated, insulated heat treatment tank equipped with a central mounted temperature logger. After the heat exchanger and before the sieve, there was also installed a temperature sensor measuring the temperature of the silage going to the heat treatment tank. The heat treatment tank was filled up with 6 m^3 of filtered silage at temperature above 85 ºC. When the tank was filled up and the temperature was above 85 ºC, the timer started to count down. If the temperature got close to a predetermined minimum set-point (e.g. 87 ºC), steam was automatically dosed into the fish silage to keep the temperature above the set-point.

2.3 Sampling

Before taking the first samples each day, pipelines and taps were flushed with 5-10 liters silage or end products. For each of 20 batches processed, 5 samples of silage and 5 of end product were taken. Silage was sampled by a tap located before the heat exchanger while end product was sampled by a tap located at the outlet pipe from the heat treatment tank. The samples were filled in 100 ml leak-proof LPDE bottles with wide neck.

Sampling, transport, receipt and storage followed ISO 7218 (Microbiology of food and animal feeding stuffs-General rules for microbiological examinations). The samples were chilled and packed in insulated containers with ice packs. Samples were sent each production day and received at the laboratory before 09:00 a.m. the next day. Analysis commenced immediately upon arrival.

Composite samples were prepared at the laboratory by mixing 10 g from each of 5 single samples of raw material or end product.

Sample material remaining after analysis was stored at -30 ºC and will be kept for at least one year.

2.4 Analysis

In addition to analysis for microbiological standards given in Regulation (EC) No 1774/2202, anaerobic sulfite-reducing bacteria were also analyzed in order to demonstrate the effect of the heat treatment on spores of naturally occurring anaerobic bacteria in the fish silage. *Salmonella* and *Enterobacteriaceae* were analyzed in individual samples while *C.perfringens* and anaerobic sulfite-reducing bacteria were analyzed in composite samples.

Salmonella **Limit of detection: 1 per 25 gram**

(NordVal no. 023; Foodproof Salmonella Detection Kit, Hybridization Probes and Foodproof Salmonella Detection Kit, 5' nuclease in combination with Foodproof Short Prep I Kit).

25 g sample were pre-enriched in Buffered Peptone Water (BPW) and sub-cultured in Brain Heart Infusion Broth (BHI). Bacterial DNA was extracted by Foodproof Short Prep I Kit (Bio-tecon Diagnostics, GmbH) and Salmonella was detected by real time PCR using Roche LightCycler 1.5 and Foodproof Salmonella Detection Kit. (Biotecon Diagnostics, GmbH).

Modification: 5 ml of 0,5 M NaOH was added to the pre-enrichment cultures before incubation, to ensure that pH was brought back from approximately 4,6 to neutrality (6,9-7,1).

Enterobacteriaceae **Limit of detection: 10 per gram**
(ISO 21528-2; Microbiology of food and animal feeding stuffs-Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count method).

Modification: None

Clostridium perfringens **Limit of detection: 1 per gram**
(ISO 7937; Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of Clostridium perfringens. Colony-count technique).

Modification: In order achieve detection limit 1 per gram, 1 g undiluted sample was analyzed in addition to ordinary analysis of the initial suspension and further decimal dilutions. The amount of medium used with undiluted samples was increased to approximately 100 ml (in 13 cm Petri dishes) to raise pH to approximately 7,0.

Anaerobic sulfite-reducing bacteria Limit of detection: 1 per gram
(ISO 15213; Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of sulphite-reducing bacteria growing under anaerobic conditions).

Modification: Same as for *C.perfringens*.

2.5 Quality assurance

Scanbio K2 AS has already an approved own-check systems in place for monitoring and surveillance of the operation. This is based on the principles of HACCP according to the Regulation (EC) No. 1774/2002 article 25. It includes identification of critical control points (CCPs), monitoring of CCPs and corrective actions to be undertaken in response to non-conformity. Routines are also in place for maintenance and calibration of critical components including thermometer, alarm and timer.

The quality system elements described in Annex 1 to this report are related to the CCPs identified in the new alternative processing method, and were adopted by Scanbio KS AS during the verification trials. The CCPs are;

- pH ≤4,0 for ≥24 hours before heat treatment
- Particle size ≤10 mm before heat treatment
- Heat treatment at ≥85 ºC for ≥25 minutes

Control of acid treatment was based on checks of Commercial Documents, summarized in the Raw Material Reception Log, and on pH measurement in samples taken from the storage tank prior to processing.

Control of particle size was based on records of daily filter integrity checks in the Filter Control Log.

Control of heat treatment was based on continuous temperature graphs from the heat treatment tank and on Shift Reports providing start and stop time for heat treatment of each batch.

The Production Log confirms, for each lot produced, that the requirements to acid treatment, particle size and heat treatment are met. The documentation referred to above was reviewed and approved by the project leader.

3 Results and discussion

3.1 Process documentation (CCPs)

pH ≤4,0 for ≥24 hours before heat treatment

By comparing the Raw Material Reception Log and the Shift Reports it was documented that the productions 12/2 and 13/2 utilized raw materials received latest 10/2 and that pH measured at the time of collection was 2,8-4,1. (pH 4,1 was measured in 1 deliverance of 2,5 m^3 of a total of approximately 500 m^3). The total volume was stored for 1-2 dg before processing, pH in samples of the total volume at start of production 12/2 and 13/2 were below 4,0.

The productions 18/2, 19/2 and 20/2 utilized raw material received latest 17/2. The raw material received 17/2 had pH 3,2 at the time of collection and had a transport time of 29 hours. pH in samples of the total volume at start of production 18/2, 19/2 and 20/2 were below 4,0.

The documentation confirmed that the fish by-products had been subject to formic acid treatment at pH below 4,0 for at least 24 hours before heat treatment.

Particle size ≤10 mm before heat treatment

The Filter Control Log confirms that the filter was undamaged and that particle size of the fish silage was ≤10 mm before heat treatment.

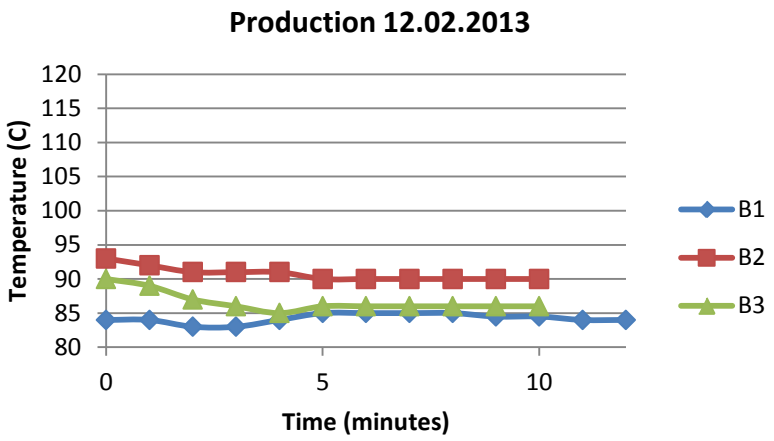
Heat treatment at ≥85 ºC for ≥25 minutes

The temperature graphs (Appendix II) and the Shift Reports show that the temperature in the heat treatment tank was ≥85 ºC for ≥25 minutes for all batches produced. The temperature sensor in the heat treatment tank was mounted in the center of the tank.

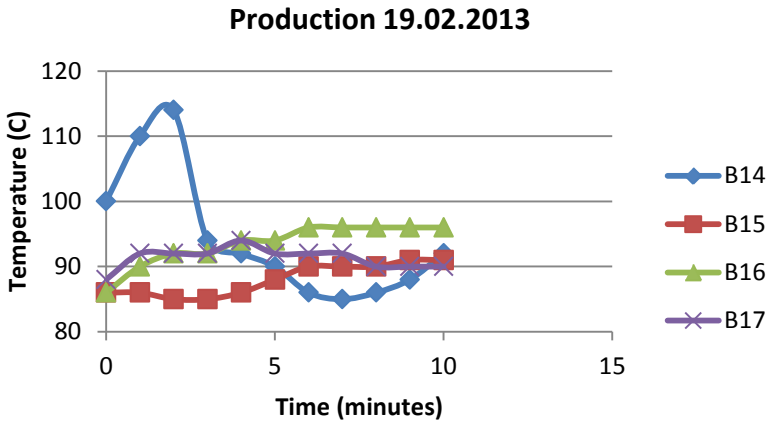
We were not able to check the temperature uniformity in the tank during heat treatment. Therefore it was decided to also read temperatures manually during emptying of the tank for some of the batches produced (Fig. 1). The temperature in the tank during heat treatment of batch 1 was at least 88 ºC, while the temperature in the outlet pipeline during emptying of the tank was occasionally below 85 ºC. Even if the observed temperature difference was probably mainly due to cooling in the outlet pipeline, the minimum temperature set-point for the tank was slightly increased during processing of batch 2-20.

Based on the data referred to above, we consider that the temperature in all parts of the 20 batches were ≥85 ºC for ≥25 minutes.

A)



B)



C)

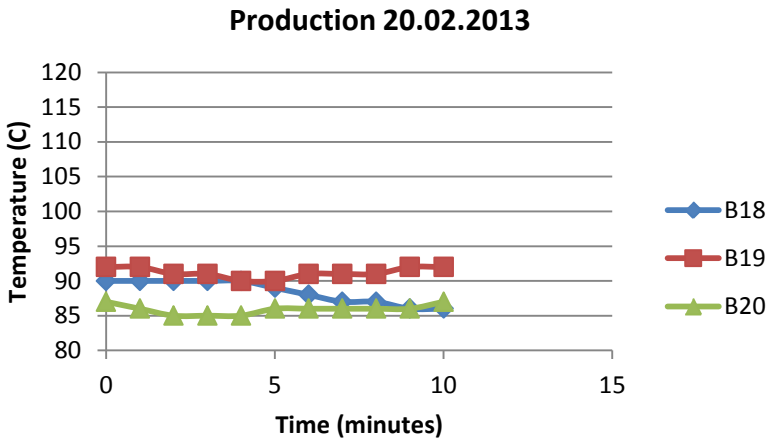


Figure 1 *Temperatures in heat treated end product measured manually in the pipeline after the heat treatment tank during emptying of the tank. A): Batch 1, 2 and 3. B): Batch 14, 15, 16 and 17. C): Batch 18, 19 and 20.*

3.2 Product documentation

Appearance

The final product is a brown liquid with a pungent odor.

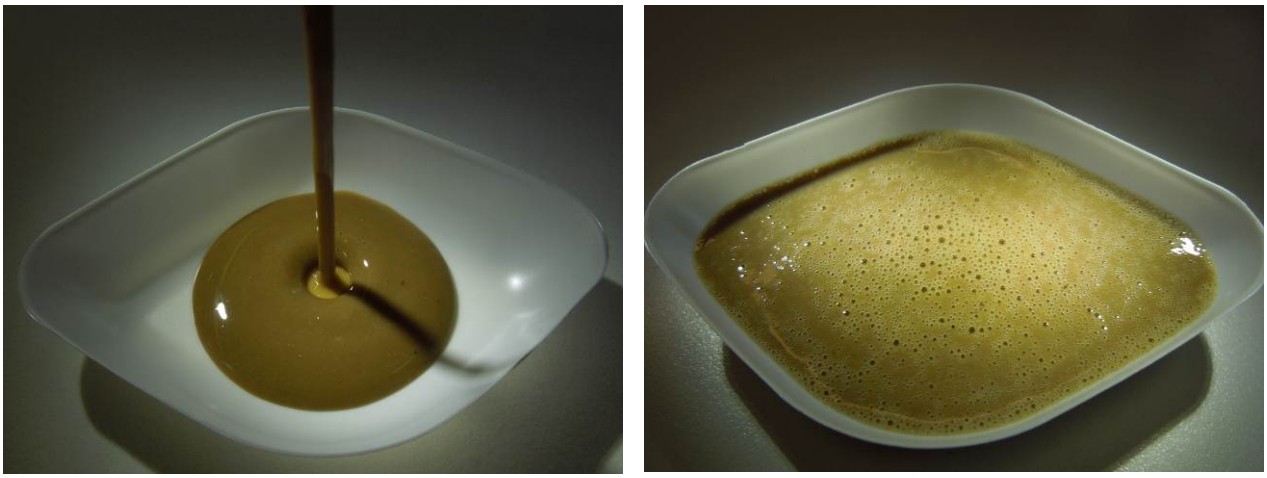


Figure 2 *The final product is a homogenous liquid. In left picture, chilled and slightly viscous end product is poured into a beaker (7,5 x 5,5 cm). In right picture room tempered end product is a thin liquid.*

pH

pH was measured in silage and end product of batch 14-20 (Table 1). The pH of the heat treated fish silage was in general the same, or slightly lower than before heat treatment, i.e. ≤4,0.

Table 1 *pH measured in composite samples of silage and end product of Batch 14-20*

Batch no.	pH Silage	pH End product
14	3,67	3,60
15	3,68	3,66
16	3,67	3,66
17	3,68	3,67
18	3,67	3,63
19	3,69	3,66
20	3,69	3,70

Bacteriological quality

Table 2 shows that *Salmonella* and *Enterobacteriaceae* could not be detected in either silage or end product. This is consistent with experimental data (Nygaard, 2009a) demonstrating that formic acid treatment of fish mince at pH 4,0 for 24 hours provided at least 4 log reductions of *Salmonella*.

C.perfringens could not be detected in either silage or end product. Also an earlier study (Nygaard, 2009b) had demonstrated the absence of *C.perfringens* in fish silage (both category 2 and 3).

Table 2 *Microbiological analysis of fish silage before and after heat treatment at Scanbio K2 AS. Salmonella and Enterobacteriaceae were analyzed in 5 samples of silage or end product, while anaerobic sulfite-reducing bacteria and C.perfringens were analyzed in composite samples.*

BATCH	PARAMETER	UNIT	#	SILAGE	END PRODUCT
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 1	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 12.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	21.000	< 1
Time: 10:47-11:12	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 2	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 12.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	24.000	< 1
Time: 12:30-12:55	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 3	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 12.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	21.000	< 1
Time: 13:55-14:20	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 4	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 13.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	2.100	< 1
Time: 08:10-08:35	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 5	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 13.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	1.100	< 1
Time: 09:52-10:17	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 6	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 13.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	1.300	< 1
Time: 11:15-11:40	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 7	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 13.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	3.000	< 1
Time: 12:20-12:45	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 8	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	4.600	4
Time: 06:25-06:50	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 9	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	3.200	< 1
Time: 07:13-07:38	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 10	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	19.000	46
Time: 08:16-08:41	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 11	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	5.900	49
Time: 09:29-09:54	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 12	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	40.000	430
Time: 10:24-10:49	*Clostridium perfringens*	Per g	1	< 1	< 1

BATCH	PARAMETER	UNIT	#	SILAGE	END PRODUCT
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 13	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 18.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	49.000	4
Time: 11:19-11:44	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 14	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 19.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	110.000	3
Time: 07:13-07:38	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 15	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 19.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	120.000	< 1
Time: 08:32-08:57	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 16	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 19.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	130.000	1
Time: 10:27-10:52	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 17	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 19.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	84.000	2
Time: 11:25-11:50	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 18	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 20.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	20.000	290
Time: 07:47-08:12	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 19	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 20.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	24.000	1
Time: 08:58-09:42	*Clostridium perfringens*	Per g	1	< 1	< 1
	Salmonella	In 25 g	5	Not detected	Not detected
Batch no. 20	*Enterobacteriaceae*	Per g	5	< 10	< 10
Date: 20.02.2013,	Anaerobic sulfite-reducing bact.	Per g	1	18.000	< 1
Time: 10:14-10:54	*Clostridium perfringens*	Per g	1	< 1	< 1

Regulation 1774/2002/EC uses *Salmonella, Enterobacteriaceae* and *C.perfringens* in samples taken directly after heat treatment as indicators to assess the feasibility and the inactivation effect of new processing methods according to method 7. The microbiological standards are summarized in table 3.

Table 3 Microbiological requirements applying to heat treated end product.

		n	c	m	M
Salmonella	in 25 gram	5	0	0	0
Enterobacteriaceae	in 1 gram	5	2	10	300
C.perfringens	in 1 gram	1	0	0	0

n: Number of samples tested
m: Threshold number of bacteria, the result is satisfactory if all samples is less than or equal to m
M: Maximum value of number of bacteria, the results are unsatisfactory if the number of bacteria in one or more samples is M or greater
c: Number of samples the bacterial count may be between m and M, while the test is still considered acceptable if the bacterial count of the other samples is M or less

The microbiological requirements are met for all 20 batches of the end product so according to those criteria the new method performs satisfactory. However, the results give no information about the effect of the heat treatment since the indicators were absent even before the heat treatment.

Anaerobic sulfite-reducing bacteria (formerly called sulfite-reducing clostridia) constitute a physiological group covering most clostridia of relevance to food and feed hygiene, including *C.perfringens,* but also non-pathogenic clostridia and even some non spore-forming bacteria. In formic acid silage at pH <4, it is assumed that viable anaerobic sulfite-reducing bacteria are mainly clostridial spores as most vegetative bacteria will be inactivated by the formic acid.

An earlier study (Nygaard, (2009b) demonstrated that *C.perfringens* were absent in fish silages, while the prevalence of anaerobic sulfite-reducing bacteria were high. This analysis parameter was included in the present study in order to give an indication to the degree of spore inactivation during heat treatment of fish silage at 85 ºC for 25 minutes.

Table 2 shows that anaerobic sulfite-reducing bacteria are abundant in the fish silage but only low numbers are present in some batches of the end product. The numbers encountered before and after heat treatment of each batch should not be compared pairwise to estimate the degree of inactivation since the spatial distribution of bacteria in natural non-homogenous matrices is uneven and the composition of the bacterial flora is variable.

If the results for all batches are seen as a whole, however, the heat treatment provided on average more than 3 log reductions which are comparable to the inactivation rates found in earlier lab-scale experiments with *C.perfringens* and *C.sporogenes* spores. In fish silage with pH 4,0 and 3,5, the same heat treatment gave respectively 2-3 and more than 6 log reduction of *C.perfringens* spores (Nygaard, 2009b). In another study where *C.sporogenes* spores were surrogate for *C.perfringens* spores, the heat treatment gave 2,8 log reductions at pH 4,0 (Nygaard and Lie, 2011) while the inactivation effect of the complete process was at least 3 log reductions.

Based on the microbiological examinations, it is concluded that the risk related to pathogens present in fish ABP from aquaculture would be adequately reduced by the proposed process.

4 References

Nygaard, H. (2009a). Inactivation of pathogenic microorganisms in fish by-products. Sub project Salmonella. Nofima Report K-354.

Nygaard, H. (2009b). Thermal inactivation of Clostridium perfringens spores in fish silage. Nofima Report K-343.

Nygaard, H & K.M. Lie (2011). Inactivation of Clostridium sporogenes spores in fish by-products by a new processing method. Nofima Report 10/2011.

APPENDIX I

New processing method for ABP category 2 materials of fish origin
Quality System Elements

to be used in verification trials at Scanbio K2 AS

February 12th - 20th 2013

Scanbio K2 AS

TABLE OF CONTENTS

HACCP-DOCUMENTATION

Introduction

A new processing method for ABP category 2 materials of fish origin was tried in full scale at Scanbio K2 AS February 12th - 20th 2013. The processing method is characterized by fish raw material that is grinded before mixing with formic acid at pH ≤4 and stored for ≥24 hours, before heat treatment of the silage with a particle size ≤10 mm at a temperature ≥85°C for ≥25 minutes.

The purpose of the verification trials was to demonstrate that the requirements of the HACCP-plan could be achieved during full scale production, that relevant pathogens are inactivated by the new processing method and that the end product is safe.

Scanbio K2 AS is approved by the Norwegian Food Safety Authority as a processing plant for category 2 materials of fish origin according to Regulation (EC) No 1774/2002.

The quality system elements described herein was used during the trials. The elements were used together with prerequisite programs, administrative routines, etc. which are part of the company's existing quality management system.

Product description

The end product of the new process is heat-treated fish silage.

The end product could be placed on the marked as such, i.e. as feed ingredient for fur animals, as substrate for biogas production, for technical use or as a fertilizer, etc.

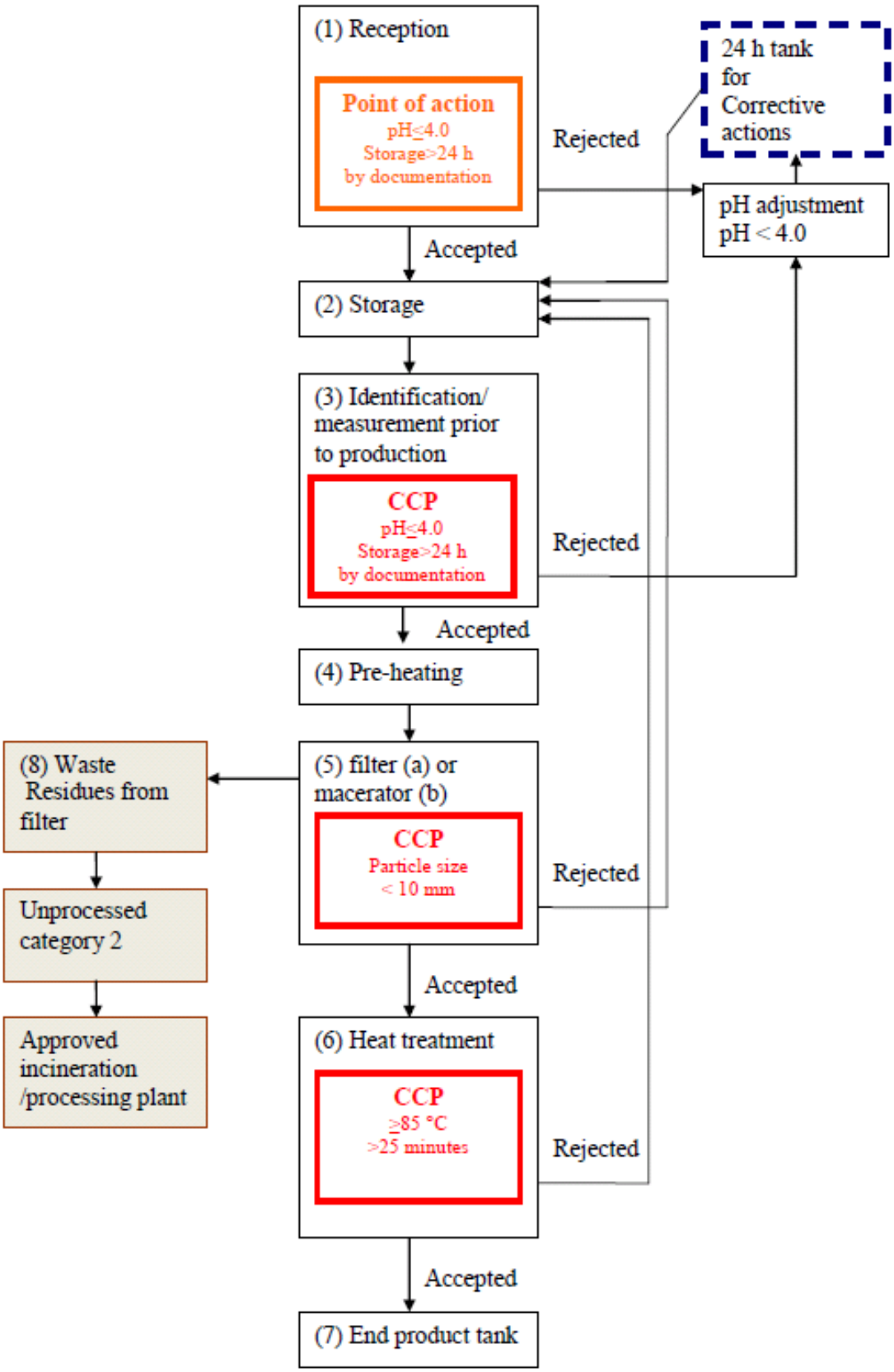
It could also be placed on the marked for further processing and separation of oil from the protein/water phase. The fish oil may be used as fuel, for technical use, fertilizer or feed ingredient for fur animals, etc. The protein containing water phase may be evaporated to decrease its water contents, resulting in a protein concentrate. The protein phase may be used as feed ingredient for fur animals etc. or as a fertilizer.

Risk assessment

EFSA considered the documentary evidence[1] provided by FHL and concluded that the risk related to pathogens present in fish ABPs from aquaculture would be adequately reduced by the described process, if the requirements of the HACCP-plan are achieved.

[1] Documentary evidence is listed in; "Scientific Opinion on the evaluation of a new processing method for ABP Category 2 materials of fish origin". European Food Safety Authority (EFSA), 2011. http://www.efsa.europa.eu/en/efsajournal/doc/2389.pdf

Process flow chart



HACCP-Plan

HACCP-plan lists all AP and CCPs together with the control measures and corrective actions to be accomplished when critical limits are exceeded

Point of action (AP)	Hazard	Critical limits	Monitoring procedures				Corrective actions	Records	Verification procedures
			What	How	When	Who			
1. Reception	Microbial survival and growth due to reduced acid exposure	**Storage at pH ≤ 4,0 for ≥ 24 h**	Document control and pH measurement	Check comm. document & pH-logg	Every shipment	Factory operator	Store for ≥ 24 h at pH ≤ 4,0	Form	Records reviewed once pr week by factory manager
Critical Control Point (CCP)	**Hazard**	**Critical limits**	**Monitoring procedures**				**Corrective actions**	**Records**	**Verification procedures**
			What	**How**	**When**	**Who**			
3. Control prior to processing	Microbial (spore) survival due to reduced synergistic effect of acid- and heat treatment	**Storage at pH ≤ 4,0 for ≥ 24 h**	Control of log and pH-measurement	Check log Measurement in sample from storage tank	1/shift	Factory operator	Adjust to pH ≤ 4,0 and store for ≥ 24 h	Form	Records reviewed once pr week by factory manager
5 A. Reduction	Core temperature not reached due to particle size > 10 mm	**Filter screen undamaged**	Integrity check of filter screen	Visual control	1/day	Factory operator	Replace/repair	Form	Records reviewed once pr week by factory manager
5 B. Reduction	Core temperature not reached due to particle size > 10 mm	**Slot segments undamaged**	Integrity check of slot segments	Visual control	1/day	Factory operator	Replace/repair	Form	Records reviewed once pr week by factory manager
6. Heat treatment	Microbial (spore) survival due to reduced thermal exposure	**Core temp ≥ 85 °C for ≥ 25 min**	Temperature-measurement Control of time	Sensors at the inlet,- in heating tank, - at the outlet Logging of time- closing and opening valves	Continuous	Automatic	Reprocessing	Data logger printout	Records reviewed once pr week by factory manager

Procedure for reception
(Process step 1)

Description

At reception, the fish silage is unloaded by pumping through pipelines into designated storage tanks. Each consignment is identified upon receipt and the Commercial Document is checked. Two "points of action" are monitored. These are not "critical control points" but of practical importance since they confirm storage time at pH ≤4.0 prior to reception, allowing for immediate processing after reception.

In general, all fish silage collected has been stored at pH ≤4.0 for ≥24 hours at the premises where it originates. At the aquaculture production site the consignor issues a Commercial Document stating pH and date of collection. Both consignor and transporter keep records of commercial documents to ensure traceability and for documentation purposes according to Regulation (EC) No 1774/2002 Annex 2.

Point of action: pH ≤4.0

Monitoring procedures: At reception, it is checked that the pH values stated in the Commercial Document are ≤4.0. pH is recorded in the Raw Material Reception Log. Commercial Documents are kept at the processing plant.

Corrective actions: If parts of the consignment had pH not ≤4.0 at the time of collection, pH of the consignment must be measured at reception. If pH of the consignment is not ≤4.0, pH must be adjusted and the consignment stored in a dedicated tank for ≥24 hours prior to further processing.

Point of action: Storage for ≥24 hours at pH ≤4.0

Monitoring procedures: At reception, it is checked that the time of collection stated in the Commercial Document is at least 24 hours in advance. Time of reception is recorded in the Raw Material Reception Log. Commercial Documents are kept at the processing plant.

Corrective actions: If parts of the consignment have not been stored for ≥24 hours before reception the consignment must be stored in a dedicated tank for ≥24 hours prior to further processing.

(During the verification trials, formerly issued Commercial Documents were used which lack boxes for registration of time of collection).

Procedure for control prior to processing
(Process step 3)

Description

Minor changes in pH are normal the first days following fish silage production. To ensure pH ≤4.0 prior to and during further processing, a sample of the total volume in the storage tank is measured prior to processing.

Critical control point:	pH ≤4.0
Monitoring procedures:	pH in a sample of the total volume in the storage tank is measured prior to processing. The result is recorded in the Production Log.
Corrective actions:	If pH >4.0, the total volume must be adjusted to pH ≤4.0 and stored for >24 hours in a dedicated tank. pH must be measured again prior to further processing.
Critical control point:	Storage for ≥24 hours at pH ≤4.0
Monitoring procedures:	The Reception Log which contains information on storage time for the fish silage in the storage tank to be processed is checked. The result is recorded in the Production Log.
Corrective actions:	If the storage time is inadequate, the content of the storage tank must be stored in a dedicated tank for ≥24 hours at pH ≤4.0 prior to further processing.

Procedure for reduction of particle size
(Process step 5 A and 5 B)

Description

Two alternative methods can be used to ensure particle size less than 10 mm. Both filter (5A) and a fine grinding macerator (5B) guarantees the required particle size. Metal filters let particles smaller than 10 mm through while particles larger are sorted out and collected in a waste container. Fine maceration is done by a macerator. This is a pump that grinds all kinds of material to a certain particle size. Manufacturers of such pumps guarantee correct particle size.

Scanbio K2 AS used the filter method (5 A). The filter was placed between the pre-heating unit (heat exchanger) and the heat treatment tank.

(Process step 5 A)

Critical control point:	Particle size <10 mm
Monitoring procedures:	Filter integrity is checked daily by operator. The result is recorded in the Filter Control Log.
Corrective actions:	Filter repair or replacement and reprocessing of batches produced after last check.

(Process step 5 B)

Critical control point:	Particle size <10 mm
Monitoring procedures:	Macerator (slot segments) is checked daily by operator.
Corrective actions:	Repair. In case of damage the process stops automatically.

Procedure for heat treatment
(Process step 6)

Description:

The fish silage is heated to a temperature above 85 °C in a pre-heating device (heat exchanger) before it is pumped to the heat treatment tank. Temperature after pre-heating is recorded in Production Log. (Y/N)

The heat treatment tank was equipped with a temperature sensor. When the tank was filled up and the temperature was above was above 85 ºC, the timer started to count down. If the temperature got close to a predetermined minimum set-point (e.g. 87 ºC), steam was automatically dosed into the fish silage to keep the temperature above the set-point.

Critical control point:	Temperature ≥85 °C for ≥25 minutes.
Monitoring procedures:	Continuous temperature measurement in the heat treatment tank. Records of temperature and holding time are kept.
Corrective actions:	If required temperature or holding time is not achieved, the material must be re-processed.

HANDELSDOKUMENT
Nr 011154

Scanbio Bjugn AS

7160 Bjugn • Telefon 72 52 07 00 • Fax 72 52 07 29

For transport av animalske biprodukter og bearbeidede produkter som ikke skal benyttes som fôrkemat i henhold til forordning (EF) nr. 1774/2002

Til transportør: Dette dokumentet skal følge produktet fra avsender til endelig mottaker.

Avsender .....

Adresse .....

Godkjennings-/registreringsnr. .....

Avsendersted .....

Mottaker .....

Godkjennings-/registreringsnr. .....

Adresse .....

Transportør, transportmiddel og identifikasjon av produkt

Transportør .....

Adresse .....

Transportmiddel (bil/skip) ..... Registreringsnr./skipsnavn: .....

BESKRIVELSE AV ANIMALSK PRODUKT

Bearbeidet biprodukt	***Best.dato/ønsket mengde***	***Ordrenr.***	***Levert tonn***
Kategori 3, Scan-pro 35/4, marint protein konsentrat	(...../.....)	.....	.....
Kategori 3, Scan-pro 31/5, fiske protein konsentrat	(...../.....)	.....	.....
Kategori 3, Scan-pro 72/2, marint protein mel	(...../.....)	.....	.....
Kategori 3, Scan-oil M, marin olje	(...../.....)	.....	.....
Kategori 3, Scan-oil F, fisk olje	(...../.....)	.....	.....
Kategori 2, Protein konsentrat	(...../.....)	.....	.....
Kategori 2, Olje	(...../.....)	.....	.....

Behandlingsmetode: Ensilert og varmebehandlet

Forseglingsnr. på prøve til avsender ☐ **Forseglingsnr. på prøve til mottaker** ☐

Merknader: .....

.....

.....

Sted ..... Dato ..... Underskrift avsender .....

Sted ..... Dato ..... Underskrift transportør .....

Sted ..... Dato ..... Underskrift mottaker .....

Dokumentet skal være utfylt i tre eksemplarer, original følger sendingen, kopi til avsender og transportør. Oppbevares i minst 2 år.

FOSEN TRYKKERI AS, BRÆKSTAD TLF 72 52 27 40

Scanbio
Telefon 72 52 07 00
Fax 72 52 07 29

TMT
A Scanbio Marine Group company
Telefon 56 14 73 00

HANDELSDOKUMENT
Nr 008701

For transport av animalske biprodukter og bearbeidede produkter som ikke skal benyttes som fôrmat i henhold til forordning (EF) nr. 1774/2002

Til transportør: Dette dokumentet skal følge produktet fra avsender til endelig mottaker.

AVSENDER .....

Lokalitetsnr. .....

Godkjennings-/registreringsnr. .....

Avsendersted .....

MOTTAKER

- ☐ Scanbio Bjugn AS, 7160 BJUGN (godkj.nr. 800094)
- ☐ Scanbio Lysøysund AS, 7168 LYSØYSUND (godkj.nr. 1000724)
- ☐ Scanbio K2 AS, 7168 LYSØYSUND (godkj.nr. 1000672)
- ☐ Miljøprosess AS, 9990 BÅTSFJORD (godkj.nr. 120857)
- ☐ Tank og Massetransport AS, 5309 KLEPPESTØ (godkj.nr. 120881 (kat. 3) og godkj.nr. 120873 (kat. 2))
- ☐ .....

Transportør, transportmiddel og identifikasjon av produkt

Transportør .....

Adresse .....

Transportmiddel (bil/skip) ..... Registreringsnr./skipsnavn: .....

BESKRIVELSE AV ANIMALSK BIPRODUKT

Kategori 3, Oppdrettsfisk *(laks, ørret, torsk)*, ensilert	..... m³	pH	Lastetid .....
Kategori 3, Gråfisk/hvitfisk *(torsk, sei o.l - villfanget)*, ensilert	..... m³	pH	Lastetid .....
Kategori 3, Pelagisk fisk *(sild, lodde)*, ensilert	..... m³	pH	Lastetid .....
Kategori 2, Selvdød oppdrettsfisk, ensilert	..... m³	pH	Lastetid .....

Hel fisk/kvernet ..... m³

Kat. 2 ☐ Kat. 3 ☐

Ensilox tilsatt ..... kg

Kjøretid Fra kl ..... Til kl ..... Sum timer .....

Lastetid Fra kl ..... Til kl ..... Sum timer .....

Har biproduktene opprinnelse fra oppdrettsanlegg/-slakteri med sykdom og/eller sykdomsrestriksjoner fra Mattilsynet?
(NB! Må alltid krysses av ved lasting av oppdrettsensilasje) ☐ Ja ☐ Nei

Levert til avsender av biproduktene ..... liter Ensilox ..... liter Antiboil

Merknader: .....

.....

.....

Sted ..... Dato ..... Underskrift avsender .....

Sted ..... Dato ..... Underskrift transportør .....

Sted ..... Dato ..... Underskrift mottaker .....

Dokumentet skal være utfylt i tre eksemplarer, original følger sendingen, kopi til avsender og transportør. Oppbevares i minst 2 år.

FOSEN TRYKKERI AS, BRØSTAD, TLF 72 52 27 40

Raw Material Reception Log

fhl	Reception	Created by:	1 of 1
		Approved by:	Revision no:
Doc.: No. 2	Raw Material Reception Log	Date:	Revision date:

Date and time of reception	Comm. Doc. number	Vessel	Consignor	Quantum m3	pH at collection	Signature

Filter control log

fhl	Production	Created by:	1 of 1
		Approved by:	Revision no:
Doc.: No. 3	Filter control log	Date:	Revision date:

Date and time	Filter in function (Y/N)	Any signs of damagesw (Y/N)	Signature

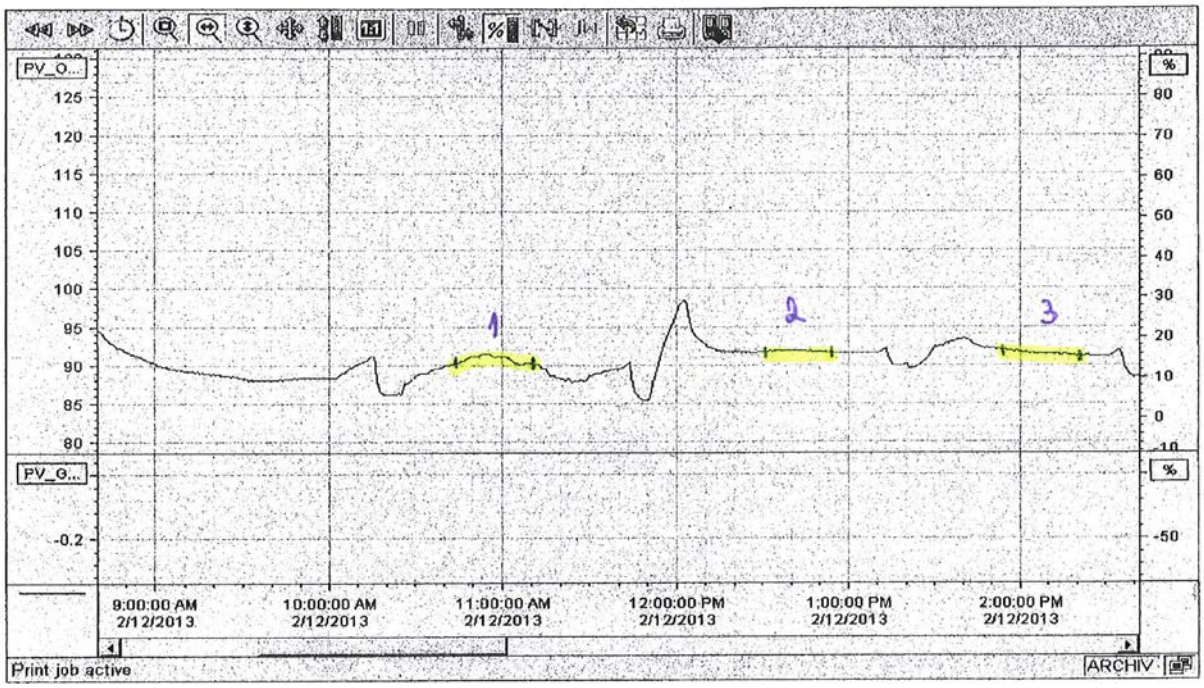
Production log

fhl	Production	Created by:	1 of 1
		Approved by:	Revision no:
Doc.: No. 3	Production log	Date:	Revision date:

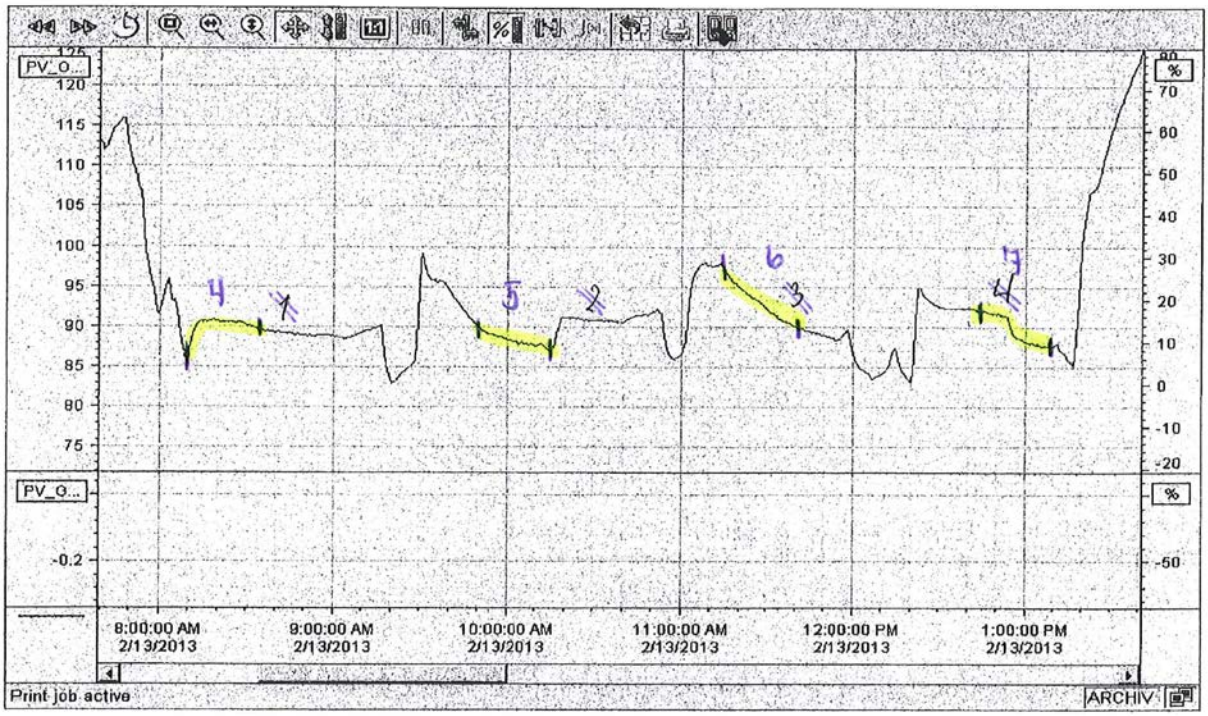
Batch ID	Date and time	Control of raw material prior to production.		Preheat and filter control		Heat treatment control		Accepted batch (Y/N)	Signature
		pH≤4.0	Stored for >24h	Preheat > 85°C	Particle size <10mm - Log of filter control checked (Y/N)	Minimum temperature in holding tank during process > 85°C (Y/N)	Minimum holding time >25 minutes (Y/N).		

APPENDIX II

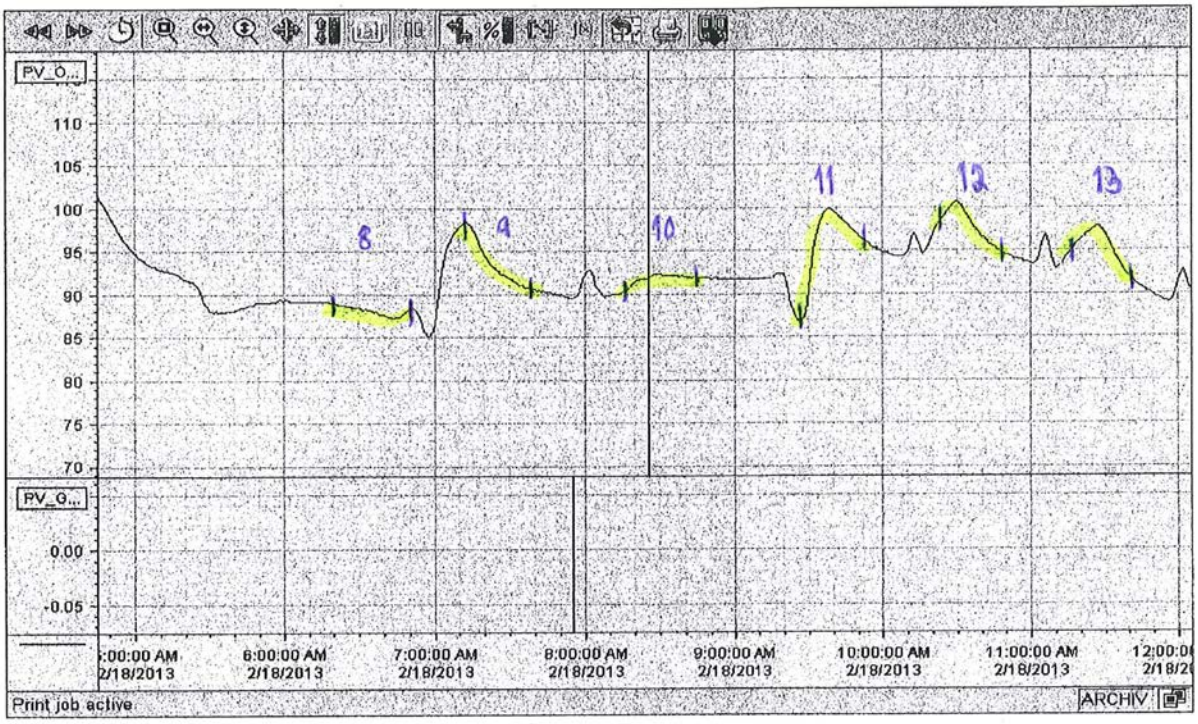
Productions 12/2 2013



Productions 13/2 2013



Productions 18/2 2013



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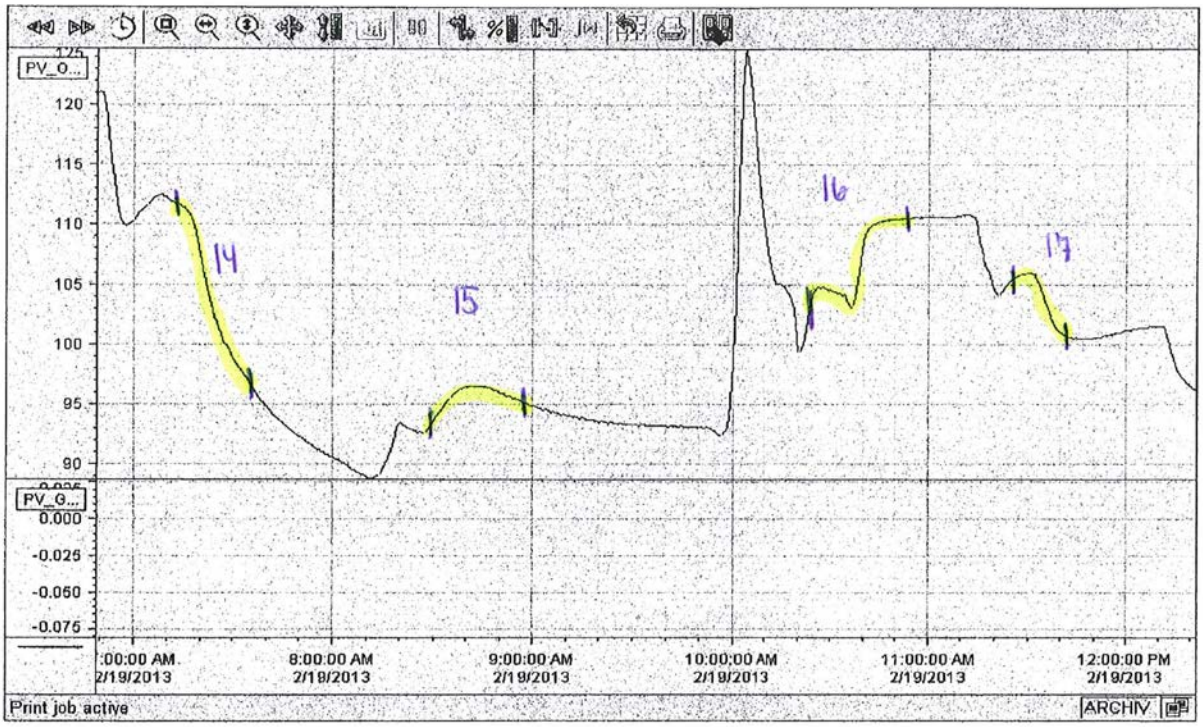
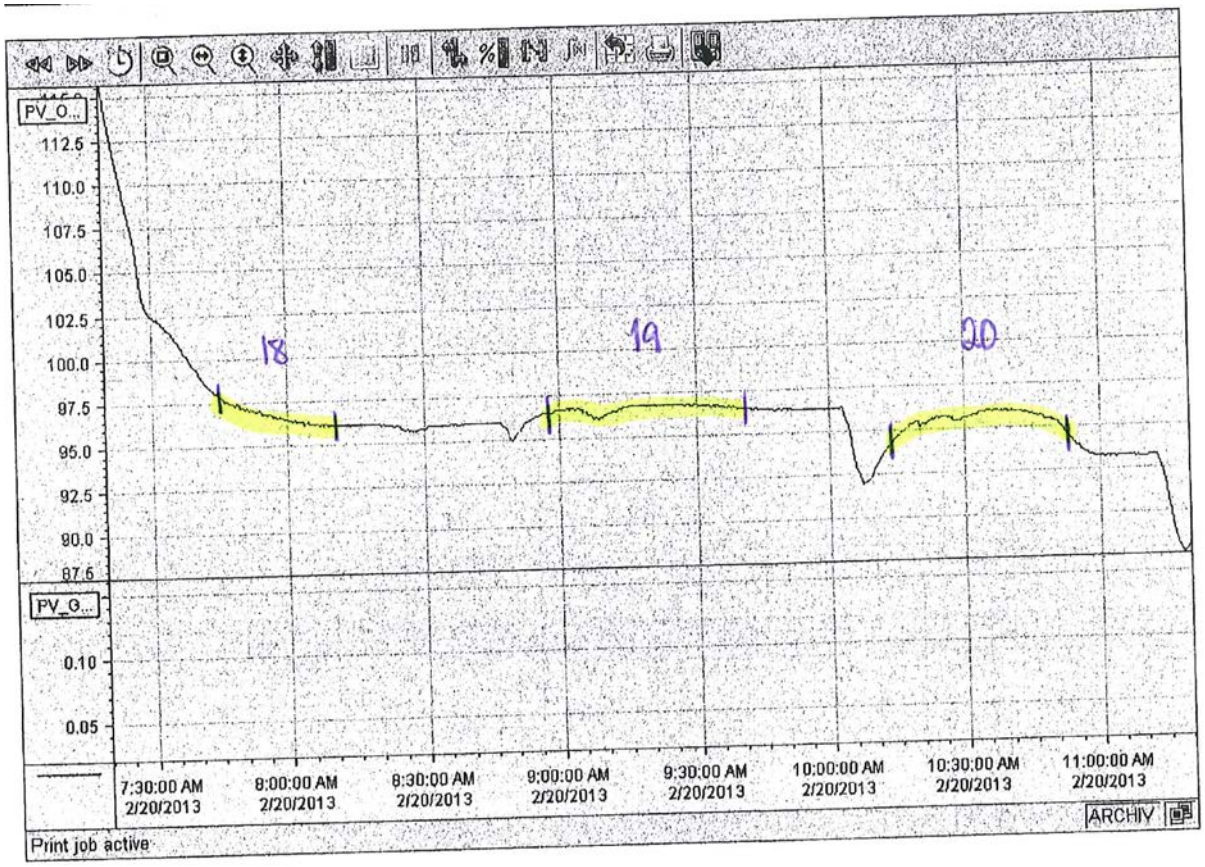


Productions 20/2 2013



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