Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Assessment of the Fish Silage Processing Method (FSPM) for treatment of category 2 and 3 material of fish origin

Opinion of the Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety

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Assessment of the Fish Silage Processing Method (FSPM) for treatment of category 2 and 3 material of fish origin

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Persons working for VKM, either as appointed members of the Committee or as *ad hoc* experts, do this by virtue of their scientific expertise, not as representatives of their employers. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed an *ad hoc* group to answer the request from the Norwegian Food Safety Authority. The members of the *ad hoc* group are acknowledged for their valuable work on this opinion.

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ASSESSED BY

The report from the *ad hoc* group has been evaluated and approved by the Panel on Animal Feed of VKM.

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SUMMARY

The Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food Safety to evaluate how a processing method hereafter referred to as the Fish Silage Processing Method (FSPM) based on heat treatment at ≥85 °C for ≥25 min at pH ≤4.0 would affect relevant microbiological hazards of category 2 and 3 material of fish origin.

The FSPM method was previously approved by the Norwegian Animal Health Authority in 1994 (Forskrift av 13. juli 1994 nr. 723). This former approval was based upon the documented results of a project financed by the Ministry of Agriculture in 1994 but did not cover the use of category 3 material of farmed fish in fish feed . EU cleared a similar method by Commission Decision 92/562/EEC (Regulation (EC) No 446/2004 repeals Commission Decisions 92/562/EEC).

The process is described in the application from the Norwegian Seafood Federation (FHL) regarding category 2 material. Fish to be treated is collected on-site on a daily basis, and subjected to ensilation at pH ≤4, usually with formic acid. The incubation time should be ≥24 h at pH ≤4 before heat treatment can be conducted. The ensilage is transported to the processing plant and is subjected to mincing leading to a particle size ≤10mm, followed by heat treatment at ≥85 °C for ≥25 min. This is followed by a filtration step and leads to the end product "Heat-treated fish silage", which may be further fractionated into fish oil, protein water and protein concentrate. Possible uses of the end-products are as agricultural fertilizers, biofuels and feed for fur/zoo/pet/circus animals. The applicant has provided reports on inactivation studies of fish pathogens, *Salmonella* sp. and *Clostridium perfringens.*

Based on the application, the Norwegian Food Safety Authority asked the Norwegian Committee for Food Safety (Animal Feed Panel, hereafter referred to as the Panel) to evaluate the potential reduction of microbiological risks as a result of the FSPM method for animal by-products of category 2 and 3 material of fish origin. An *ad hoc* group was appointed to perform this task.

The Panel concludes that the FSPM method will inactivate non-spore-forming bacteria, *Clostridium perfringens*, moulds, *Saprolegnia*, parasites and viruses in category 2 and 3 material of fish origin.

For category 2 and 3 material from fish cultivated in marine waters in net cages where dead fish are removed on a daily basis, and for category 3 material for fish cultivated in earth ponds, the levels of *C. botulinum* will be low, and the FSPM method will inactivate *C. botulinum* present.

For category 2 material from fish cultivated in earth ponds where dead fish are not removed on a daily basis, the concentration of *C. botulinum* is reported to reach levels at which the possibility of spores of *C. botulinum* type E surviving heat treatment cannot be ruled out. The spores surviving heat treatment will not germinate as long as a pH ≤4 is maintained. Preformed toxins of type E will be destroyed by the FSPM method.

The FSPM method will degrade DNA; thus genes potentially encoding antibiotic resistance will be inactivated.

The FSPM method will not inactivate mycotoxins. However, it has not yet been shown that residues of mycotoxins in fish pose any hazard which demands particular treatment concerning animal or human health.

The FSPM method will not inactivate potential prions in by-products from fish. However, it is unlikely that prions from fish pose any hazard to animal or human health.

Norsk sammendrag

Mattilsynet har bedt Vitenskapskomiteen for Mattrygghet om å vurdere hvordan en prosesseringsmetode (heretter omtalt som FSPM-metoden (FiskeEnsilasje BearbeidingsMetode) basert på varmebehandling ved ≥85 °C i ≥25 min ved pH≤4.0 vil innvirke på relevante mikrobiologiske farer for kategori 2 og 3 materiale fra fisk.

FSPM metoden var tidligere godkjent av Dyrehelsetilsynet i 1994 (Forskrift av 13. juli 1994 nr. 723), mens bruk av oppdrettsfisk til oppdrettsfisk var utelukket. Denne godkjenningen var basert på dokumenterte resultat fra et prosjekt finansiert av Landbruksdepartementet i 1994. EU har åpnet for en lignende metode ved kommisjonsvedtak 92/562/EEC (Regulering (EC) No 446/2004 tilbakekaller kommisjonsvedtak 92/562/EEC).

Prosessen for bearbeiding av kategori 2 materiale er beskrevet i søknaden fra Fiskeri- og Havbruksnæringens Landsforening (FHL). Fisk som skal behandles oppsamles daglig, og ensileres ved pH ≤4, vanligvis med maursyre. Inkubasjonstiden skal være ≥24 t ved pH ≤4 før varmebehandlingen kan gjennomføres. Ensilasjen transporteres til prosesstedet og blir hakket, noe som fører til en partikkelstørrelse på ≤10mm, fulgt av varmebehandling ved ≥85 °C i ≥25 min. Dette etterfølges av et filtreringstrinn og gir sluttproduktet "Varmebehandlet fiskeensilasje". Mulig bruk av sluttproduktet er som gjødsel, bioenergiproduksjon, og fôr til pels-, kjæle- og sirkusdyr. Søkeren har fremskaffet rapporter på inaktiveringsstudier for fiskepatogener, *Salmonella* og *Clostridium perfringens*.

Basert på søknaden, ba Mattilsynet Vitenskapskomiteen for mattrygghet (Faggruppen for fôr til terrestriske og akvatiske dyr, heretter omtalt som Faggruppen) om å vurdere potensiell reduksjon av mikrobiologisk risiko som en følge av behandling med FSPM-metoden for kategori 2 og 3 biprodukter fra fisk.

En *ad hoc*-gruppe ble oppnevnt for å arbeide med dette oppdraget. Faggruppen behandlet rapporten på sitt møte 2 desember 2009 og ga da sin tilslutning til rapporten fra *ad hoc*-gruppen.

Faggruppen konkluderer med at FSPM-metoden vil inaktivere ikke-sporedannende bakterier, *Clostridium perfringens*, sopp, *Saprolegnia*, parasitter og virus i kategori 2 og 3 materiale fra fisk.

For kategori 2 og 3 materiale fra sjøbasert fiskeoppdrett hvor død fisk fjernes på daglig basis, og for kategori 3 materiale for fisk oppdrettet i jorddammer, vil nivået av *C. botulinum* være lavt, og FSPM-metoden vil inaktivere tilstedeværende *C. botulinum*.

For kategori 2 materiale fra fisk oppdrettet i jorddammer hvor død fisk ikke fjernes daglig, er det rapportert at konsentrasjonen av *C. botulinum* kan nå nivåer slik at det ikke kan utelukkes at sporer av *C. botulinum* type E kan overleve varmebehandlingen. Sporer som overlever varmebehandling vil ikke germinere så lenge pH ≤4. Preformerte toksiner av type E vil bli inaktivert av FSPM-metoden.

FSPM-metoden vil degradere DNA; slik at potensielle antibiotika resistensgener vil bli inaktivert.

FSPM-metoden vil ikke inaktivere mykotoksiner, men det er ikke dokumentert at mykotoksiner i fisk utgjør noen helserisiko som krever spesiell forbehandling, for dyr eller mennesker som spiser fisken.

FSPM-metoden vil ikke inaktivere potensielle prioner i biprodukter fra fisk, men det er usannsynlig at prioner fra fisk vil utgjøre noen helserisiko for dyr eller mennesker.

BACKGROUND

The FSPM method

The Norwegian Seafood Federation has (at 11/17/2008) applied to the Norwegian Food Safety Authority (NFSA) for approval of the FSPM method for treatment of category 2 products of fish from aquaculture. The method includes heat treatment at minimum 85 °C for at least 25 min and at a pH of ≤ 4. The applicant has provided reports on inactivation studies of fish pathogens and *Salmonella* sp. and *Clostridium perfringens.*

A method based on the same principle has previously been approved by the Norwegian Animal Health Authority in 1994 (Forskrift av 13. juli 1994 nr. 723). This approval was based upon the documented results of a project financed by the Ministry of Agriculture in 1994. A similar method was cleared by Commission Decision 92/562/EEC (Regulation (EC) No 446/2004 repeals Commission Decisions 92/562/EEC).

Description of the process

The process of the FSPM method is described in the application from The Norwegian Seafood Federation (FHL). Fish to be treated is collected on-site at a daily basis, and subjected to ensilation at pH ≤4, usually with formic acid. The incubation time should be≥24 h at pH ≤4 before heat treatment can be conducted. The ensilage is transported to the processing plant and is subjected to mincing leading to a particle size ≤10mm, followed by heat treatment at ≥85 °C for ≥25 min. This is followed by a filtration step and leads to the end product “Heat treated fish silage”, which may be further fractionated into fish oil, protein water and protein concentrate. Possible uses of the end-products are as agricultural fertilizers, biofuels and feed to fur-, zoo-, pet and circus animals.

Based on the application the Norwegian Food Safety Authority asked the Norwegian Committee for Food Safety (Animal Feed Panel) to evaluate the potential reduction of microbiological risks as a result of the FSPM method for category 2 and 3 by-products of fish origin. An *ad hoc* group was appointed to perform this task.

Legal background

Regulation (EC) No 1774/2002 lays down animal and public health rules for the collection, transport, storage, handling, processing and use or disposal of animal by-products, to prevent these products from presenting a risk to animal or public health. The 1774/2002 regulation is implemented in Norway through the animal by-product regulation (NO) 2007-10-27 nr 1254.

The 1774/2002 regulation divides animal by-products into three different risk categories 1, 2 and 3, determining and limiting their use and disposal. Category 1 is most risky.

Animal by-products of Category 1 material is in general disposed of as waste by incineration. Category 2 material shall be used as Category 1 material or processed for technical use or fed to animals outside the food chain as specific users according to derogations. Category 3 material may after processing and in general be fed to food producing animals. The categorization of animal by-products is not always clear cut.

Dead and clinically ill fish with outer signs of disease are Category 2 material. In general animal by-products originating from slaughtering of fish for human consumption is Category 3 material.

Although not clinically ill, fish killed for disease control purposes can be considered Category 2 material. In practice, and in accordance with Community legislation, such fish may in the course of disease control operations be harvested for human consumption, as IHN, VHS and ISA are diseases not harmful to humans. The by-products from plants manufacturing such fish products for human consumption are considered as Category 3. However such fish and its by-products may present an animal health risk to fish and shall not be used in feed for fish. The ruling NFSA (Norwegian Food Safety Authority) guidance and interpretation of regulation is that Category 3 material both fish meal and fish oil from aquaculture farms with proven list 1-3 diseases, shall not be used in feed to fish

The regulation 1774/2002 restricts the feeding of a species with processed animal protein derived from the bodies or parts of bodies of animals of the same species. Derogations have been granted in relation to wild fish and fur animals by EU comitology procedure, after consultation of the appropriate scientific committee.

Until recently animal by-products of aquaculture origin could not be fed to farmed fish in Norway. This is mainly why processing methods for such use have not been developed and documented by the industry yet. The same goes for controls and interpretation of the intra-species recycling ban for fish.

In annex V the 1774/2002 regulation predefines 7 processing methods. Method 1 is a reference comprising heat treatment at 133 °C at 3 bar overpressure for 20 minutes. Today method 1 is the only applicable processing method for Category 2 material of fish origin in EU. Member States (EU/EFTA) may demand use of method 1 prior to dispatch before receiving Category 1 and 2 material and processed animal protein (fish meal) form other member states. However, 1774/2002 allows for the adaptation of an alternative method for the processing of category 2 material of fish origin through the comitology procedure. The NFSA has therefore asked the Norwegian Committee for Food Safety (VKM) for an evaluation of the applied processing method with a view to making the method a part of the EU legislation.

Norway permits the applied processing method (FSPM) (≤ pH 4 > 85 °C in > 25 minutes) because it was permitted in former regulation for use for high risk waste (Category 2 material) and low risk waste (Category 3 material). Although permitted for Category 3 material, the FSPM method was not risk assessed and documented for the use of by-products from aquaculture in feeds to aquaculture animals. This is why NFSA (Norwegian Food Safety Authority) asks the VKM for a risk assessment of processing Category 3 material using the FSPM method, in addition to processing Category 2 material.

If NFSA confirms that the FSPM processing method is safe, it can be approved nationally as a method 7 for both fish meal and fish oil providing that all other regulatory demands are fulfilled.

TERMS OF REFERENCE

The Norwegian Food Safety Authority asks the Norwegian Committee for Food Safety (VKM):

To assess how the described processing method will affect relevant microbiological risks for category 2 and 3 material of fish origin

ASSESSMENT

Presumptions

The assessment is based on the presumption that the heat treated material:

- has a core temperature of at least 85 °C for at least 25 min
- pH is 4.0 or less after acidification.
- Formic acid or another organic acid is used to obtain the desired pH.

As the particle size is described to be as low as <10mm, it is assumed that these presumptions are achieved. Furthermore, it is presumed that there is no contamination with the relevant microbiological hazards after the treatment has started.

Hazard identification

The materials assessed are category 2 and 3 material of fish origin. Any hazard derived from those materials should be considered. The assessment is considering both material from fisheries and aquaculture. The materials to be treated may include infected and dead fish. Fish pathogenic viruses, bacteria, parasites and fungi are assessed. Also hazards that may be associated with infected or dead fish and which may affect animal or human health are assessed. This includes *Listeria monocytogenes,* clostridia*, Salmonella* and other *Enterobacteriaceae*. Toxins produced by microorganisms can also be present. The spread of antibiotic resistance genes is also included. The available information on prions in fish is scarce. However, prions are assessed because the inactivation of these agents is an issue in the animal by-product regulation.

Hazard characterization and risk assessment of the identified hazards

Viruses

Viruses infecting fish may be divided into different groups depending on their nucleic acid composition (DNA or RNA), and the presence or absence of an external protein-lipid capsid envelope. The virus causing Infectious pancretic necrosis (IPNV) is a non-enveloped RNA virus, and has been shown to be among the viruses being most resistant to environmental stress (EC, 2003). Inactivation of IPNV is dependent on the amount of virus, time, temperature and pH in the material to be treated. Smail *et al.*(1993) showed inactivation of IPNV (titre 8.3 $\log_{10}$ pfu/ml) in fish ensilage after 71 days at 20 ˚C, while natural amount of virus (2-2.5 $\log_{10}$ pfu/ml) was inactivated after 3 hours at 45˚C and after 1 hour at 60 ˚C. Whipple and Rohovec (1994) showed inactivation of IPNV in fish silage after 5 min at 82 ˚C, with 15 min preheating at 65 ˚C. The same work shows inactivation of IPNV in PBS after 8 hours at 60 ˚C, 3-4 hours at 65 ˚C, 2 hours at 70 ˚C and after 10 min at 80 ˚C. O.I.E. Aquatic Animal Disease Card (2000) refers to 99.999 % inactivation of IPNV after 30 min at 60 ˚C, pH 3.

The Panel concludes, based on the results evaluated for IPNV, and the knowledge that IPNV is among the most resistant viruses, that the FSPM method will inactivate viruses in category 2 and 3 material of fish origin.

Bacteria

Non-sporeforming bacteria

Heat resistance of non sporeforming bacteria is dependent on a number of factors, e.g. species, strain, physiological state, and the matrix in which the bacterium is found (Doyle and Mazzotta, 2000). The efficiency of heat treatment is considerably influenced by the constituents of the matrix, especially fats (Doyle and Mazzotta, 2000; Juneja and Eblen, 2000), available water levels (Liu *et al.*, 1969; Farkas, 2001), level and homogeneity of contamination, the temperature profile achieved through the batch and individual particles, and the minimum treatment period (Ricke, 2005). Experimental work has also shown that prior exposure to stresses such as alkaline or acid conditions may increase the heat resistance of organisms (Humphrey, 1990; Humphrey *et al.*, 1991; Farber and Pagotto, 1992; Humphrey *et al.*, 1993; Casadei *et al.*, 2001). Furthermore, the efficiency of treatments may be over-estimated in commercial conditions by ineffective sampling and testing (Williams, 1981) and the long tail of surviving organisms in low numbers which may be difficult to detect (Doyle and Mazzotta, 2000).

Consequently, predicting the effect of a combination of acid and heat treatment, as outlined in the FSPM method, on a bacterial species in a specified matrix (category 2 or 3 material of fish origin) can be difficult without data from experiments designed for this purpose. In the Nofima report (2009 a), experimental data on the effect of the FSPM method on a *Salmonella* Senftenberg strain inoculated in fish suspension are provided. Such experimental data are not available for other *Enterobacteriaceae*, *Listeria monocytogenes* and *Mycobacterium* sp. However, these bacteria were also considered in the scientific opinion of the Panel on biological hazards, Microbiological risk assessment in feedingstuffs for food-producing animals (EFSA, 2008). The Panel has taken this opinion into consideration together with relevant scientific literature.

Salmonella

Bacteria in the genus *Salmonella* are important enteropathogens, which may cause diseases ranging from mild gastroenteritis to systemic infections in humans and animals. The type of disease is determined by the virulence characteristics of the *Salmonella* strain as well as by the host species. The bacteria are mainly transmitted via the faecal-oral route, and the dose of infection may vary from 10^1 to 10^6 bacterial cells. Salmonellosis is one of the most common and widely distributed foodborne diseases in humans. According to the WHO, millions of human cases are reported worldwide every year, and the disease results in thousands of deaths (http://www.who.int/mediacentre/factsheets/fs139/en/index.html).

Salmonella spp. are Gram-negative, flagellated and facultative anaerobic bacteria. The taxonomy of *Salmonella* is rather complicated, and has been a subject of scientific debate for many years. Detailed phylogenetic analyses by multilocus enzyme electrophoresis and DNA sequencing have demonstrated that the genus *Salmonella* includes the two species *Salmonella bongori* and *Salmonella enterica,* where the latter has been further divided into at least six subspecies. Furthermore, differences in the lipopolysaccharide layer and flagella structure generate the antigenic variation that is reflected in the more than 2500 known serovars. More than 99% of the serovars responsible for disease in humans, warm-blooded animals and birds are members of subspecies I (subsp. *enterica*). Consequently, only serovars of *Salmonella enterica* subspecies *enterica* (hereafter called *Salmonella)* will be considered in this risk assessment.

Salmonella are able to survive and multiply in environments other than the intestine (D`Aoust, 1997). The bacteria may grow in the temperature range between 5 and 46 °C. Under suboptimal conditions, e.g. at temperatures below 30°C, *Salmonella* tend to form biofilms on both inert and organic surfaces. In this state, bacteria are better protected against environmental stresses (Møretrø *et al.,* 2009; Vestby *et al.,* 2009).

Salmonella have not generally been regarded as fish pathogens. Experiments have shown that even after administration of very high doses of *Salmonella*, Atlantic salmon did not exhibit any signs of disease (Nesse *et al*., 2005). However, fish may be exposed to *Salmonella* spp. through consumption of contaminated feed or by residing in contaminated water, and carry *Salmonella* in the gastrointestinal tract for several weeks depending of the dose (Nesse *et al*., 2005).

Salmonella are rarely found in Norwegian seafood. However, *Salmonella* may be found in fish meal. In the years 2000-2004, 0.25 % of domestic fish meal samples and 1.33 % of imported fish meal samples were positive for *Salmonella* (VKM, 2009). The source of contamination is mostly unknown, but one theory is that fish meal is cross contaminated after heat treatment either from the raw material or from other sources. An investigation of Norwegian fish meal in 1999-2001 showed that 8% of the batches tested were contaminated, and the level of contamination pr batch was less than 5 MPN pr 100g (Nesse, 2005). Category 2 material from fish may get contaminated by *Salmonella* from the fish itself and by cross contamination by *Salmonella* from the production environment. Levels of potential *Salmonella* contamination are not known, but it is reasonable to believe that they are relative low; possibly of the same magnitude as fish meal contamination.

Salmonella in organic material with a high water activity ($a_w > 0.97$) are readily destroyed by heat. Temperatures in the range of 80-85 ºC for 30 minutes have been reported to eliminate all *Enterobacteriaceae* from feed meal (Kampelmacher *et al.*, 1965). Furthermore, 85 ºC for one

minute is considered a good target requirement for *Salmonella* elimination in feed (Mossel *et al.*, 1967; Liu *et al.*, 1969; Jones and Richardson, 2004). In the Nofima report (2009 a) predicted D-value (Decimal reduction time) at 85°C was estimated to be 0.0001 minute.

The fish suspension tested in the Nofima report had a high content of fat (54%) and a low content of protein and ash. According to the report the composition of this fish suspension was not to be very different from industrial silages after bone sedimentation (Nofima 2009 a). Thus, it is reasonable to believe that heating this material to 85 ºC for as long as 25 minutes will eliminate realistic concentrations of *Salmonella*.

As *Salmonella* may grow in a pH range from 3.8 to 9.5 and at water activities above 0.94 (Bell, 2002), the Panel was concerned whether 24 hours in fish silage at pH 4 might increase the heat resistance of the bacteria. However, results from the Nofima report demonstrated that adding formic acid to pH 4.0 - 4.1 for 24 hours gave least 4 log reduction (no *Salmonella* was detected). Consequently, there is no reason to believe that 24 hour at pH 4 by formic acid will increase the heat resistance of *Salmonella*.

The Panel concludes that the FSPM method will inactivate Salmonella *in category 2 and 3 material of fish origin.*

Other *Enterobacteriaceae*
Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. A number of species can cause disease in humans and animals. *Escherichia coli*, better known as *E. coli*, is one of the most important model organisms, and its genetics and biochemistry have been closely studied. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicaemia and Gram-negative pneumonia (Todar, 2009).

Members of the *Enterobacteriaceae* are rod-shaped, and have Gram-negative stains. They are facultative anaerobes, fermenting sugars to produce lactic acid and various other end products. Most have many flagella used to move about, but a few genera are non-motile. They are non-spore forming.

Until 1966, *E. coli* was thought to survive only in warm-blooded animals such as birds and mammals but it has since been discovered in the intestines of wild fish. Investigations have shown fish also to be carriers of a number of other species (Acoachere *et al.,* 2009), and this is probably true for most of the species in this family. The source of these bacteria in fish is polluted water and food, indicating that fish in coastal areas to some extent will reflect the bacterial flora of the humans and fauna on nearby land. The Nofima report (2009 a) found the number of *Enterobacteriaceae* to be 1500 pr gram in their test material, which probably reflects the contamination level of the category 2 material of fish origin in general. The levels of pathogenic *Enterobacteriaceae* are probably much lower, and possibly even lower than those of *Salmonella*.

According to EFSA (European Food Safety Authority) (EFSA, 2008), comparative studies suggest that heat treatment processes used to successfully control *Salmonella* contamination will also be effective for other non spore-forming food-borne pathogens. As an example it has been reported that heat processing conditions which were appropriate for elimination of *S.*

Senftenberg and *S.* Anatum (i.e. D-value at 80 ºC at a_w of 0.8 – 12.3 minutes) could also eliminate *Yersinia pseudotuberculosis* (<0.3 mins) and *E. coli* O157:H7 (6.3 mins) (Cooke, 2002). Reduction profiles for *Salmonella* and *E. coli* O157:H7 were also similar in meat sausages (McCormick *et al*., 2003). Acid tolerance varies within *Enterobacteriaceae*, but is generally believed to be of the same magnitude as for *Salmonella*.

The Panel concludes that the FSPM method will inactivate Enterobacteriaceae *in category 2 and 3 material of fish origin.*

Listeria monocytogenes

Listeria monocytogenes is one of the most virulent foodborne pathogens with 20 percent of clinical infections among humans resulting in death. Listeriosis is thus the leading cause of death among foodborne bacterial pathogens with fatality rates exceeding even *Salmonella* and *Clostridium botulinum* (Dharmarha, 2008). It is the third most common cause of meningitis in newborns. Clinical diseases due to *L. monocytogenes* are even more frequently recognized by veterinarians, especially as meningo-encephalitis in ruminants.

The genus *Listeria* includes 6 different species, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* which can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *L. monocytogenes* is a Gram-positive bacterium, motile via flagella at 30 °C and below but usually not at 37 °C (Gründling *et al.*, 2004). Studies suggest that up to 10 % of human gastrointestinal tracts may be colonized by *L. monocytogenes* (Ramaswamy and Cresence, 2007).

The incidence of *L. monocytogenes* was shown to be higher in the Norwegian aquaculture industry, compared to samples from the pelagic industry. In the aquaculture industry; 10 % of the investigated fish (n=50) were contaminated, while in the pelagic industry, there was no *L. monocytogenes* contamination in fish or fish products. In the pelagic industry only 1.0 % of the total samples (including environmental samples) contained *L. monocytogenes* compared to 3.3 % positive for *L. monocytogenes* of the total samples from the aquaculture industry (Tvedt 2009). The overall frequency of *L. monocytogenes* contamination in the examined fish processing factories was 20.5 % (62 of 303 samples), of which raw fish, processing line samples, finished products, and environmental samples accounted for 33.3 % (6 of 18), 23.1 % (12 of 52), 7.4 % (2 of 27), and 20.4 % (42 of 206), respectively (Thuy *et al*., 2008).

It has been shown that if food contaminated with *L. monocytogenes* is exposed to mild heat or acid conditions, stress induced tolerance may occur within 1.5 h. More specifically, stress tolerance was evident by the fact that *L. monocytogenes* cultures, which were heat-treated at 46 °C and/or acid-treated at pH 5 for 0.5, 1.0 or 1.5 h, survived at 1–1.5 $\log_{10}$ CFU ml^{-1} higher level during subsequent heating at 57 °C for 50 min compared to cultures that received no pretreatment (Skandamis *et al.*, 2008). D-values of *L. monocytogenes* at 65/66 ºC varied between 0.28 to 1.19 min in salmon with a fat content of 10-17% (Doyle, 2001). These were 1.5 to 4.4 times higher than those of cod at the same temperatures, and this was explained by the much lower fat content of cod (0.5-0.8 %). Although the material from fish tested in the Nofima report had a high fat content (54 %), the high temperature and long treatment time in the FSPM method should be sufficient to eliminate realistic levels of *L. monocytogenes*. This is supported by experiment showing that heat processing conditions which were appropriate for elimination of *S.* Senftenberg and *S.* Anatum, *S.* Enteritidis and *S.* Typhimurium DT104,

could also eliminate *L. monocytogenes* (Cooke, 2002). Other studies applying heat treatment to beef, chicken meat and skin also found *L. monocytogenes* was more easily eliminated than *Salmonella* (Juneja, 2003; Murphy *et al*., 2004). Reduction profiles for *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 were similar in meat sausages, but *Listeria* was more heat sensitive than *Salmonella* in turkey Bolognese (McCormick *et al*., 2003).

The Panel concludes that although it is difficult to predict exactly how the FSPM method will affect L. monocytogenes *without proper experimental data, the Panel believes that the FSPM method will inactivate* L. monocytogenes *in category 2 and 3 material of fish origin.*

Mycobacterium
Tuberculosis, caused by *Mycobacterium tuberculosis*, is the leading cause of death worldwide from a bacterial infectious disease. In addition, other mycobacteria cause disease in both humans and animals including fish.

The genus *Mycobacterium* is currently the only genus in the family *Mycobacteriaceae*. As of June 2008, 130 *Mycobacterium* species and 11 subspecies were recognized by the *list of prokaryotic names with standing in nomenclature* (LPSN) (Euzéby, 1997). They are divided into slowly growing (require more than seven days to produce visible colonies) and rapidly growing mycobacteria (produces visible colonies in less than seven days). Nontuberculosus mycobacteria encompass all mycobacteria other than the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* (Jarzembowski and Young, 2008). The risk of fish carrying bacteria of the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* is very small, probably negligible. Consequently, only nontuberculosus mycobacteria will be considered in this risk assessment.

Mycobacterial species such as *M. marinum, M. chelonae, M. fortuitum, M. neoaurum, M. simiae, M. scrofulaceum, M. montefiorense, M. poriferae, M. shottsii* and *M. pseudoshottsii* are all potential agents of fish disease. *M. marinum* was originally isolated and identified from marine fish at the Philadelphia Aquarium (Aronson, 1926). It was initially thought to infect marine fishes only, and was named accordingly, but is now known to be a ubiquitous species. Mycobacteriosis is one of the most common diseases affecting cultured and wild fishes worldwide, including Atlantic salmon, and mycobacterial infections have been described in freshwater and marine fishes from tropical to subarctic latitudes. Use of non-pasteurised feed in hatcheries during the late 1950s promoted an increase in prevalence of fish mycobacteriosis (Parisot and Wood 1960; Ross and Johnson 1962).

M. marinum is closely related to the *Mycobacterium tuberculosis* complex and causes a disease in fish and amphibians with pathology similar to tuberculosis. Transmission of mycobacteria in fishes is poorly understood. Water and associated biofilms are natural habitats for *Mycobacterium* spp. including *M. marinum*, *M. fortuitum*, and *M. chelonae*, so waterborne transmission seems likely. Mycobacteria are known to infect a number of aquatic organisms other than fishes and survive and replicate within various protozoan hosts, so vectors are potentially present throughout the food web. Fishes are believed to be infected by ingestion of contaminated food and water. Vertical transmission of mycobacteria has been suggested, and transovarian transmission in live bearing fishes has been reported.

In addition to their known infectivity to fishes, marine mycobacteria pose significant zoonotic concerns. *M. marinum* is a well-known human pathogen, producing granulomatous lesions in skin and peripheral deep tissues (Lewis *et al.*, 2003; Petrini, 2006). However, there are reports

of generalized (systemic) infections caused by both *M. marinum* and *M. chelonae* in humans with weak immune systems. Infection with *M. marinum* produces cross-reactivity to *M. tuberculosis* PPD- and *M. avium* sensitin-based skin tests.

Mycobacterium avium complex (MAC) includes *Mycobacterium avium* subspecies *avium* (MAA), *Mycobacterium avium* subspecies *hominis* (MAH), and *Mycobacterium avium* subspecies *paratuberculosis* (MAP). *M. avium* has been recovered from almost every environmental compartment that has been investigated, including those that bring the organism into contact with susceptible species such as humans and animals including fish, such as natural water, drinking-water, biofilms, aerosols, soils, foods, plants and plant products, and fish (Falkinham, 2004).

Mycobacterium avium has been reported to persist in media at 4 to 42 °C and grow at 14-37 °C, (Archuleta *et al*., 2002), whereas *M. marinum* usually does not grow in the laboratory at 37 °C, but prefers 30-33 °C. *M. avium* is naturally tolerant to pH<3, and pre-adaptation under conditions similar to the conditions where *M. avium* is found in the environment, results in increased acid resistance. Experiments on the *Mycobacterium avium-Mycobacterium intracellulare* complex organisms in aqueous suspension showed a D-value at 70ºC of 1.5 minutes or less. Kill rates were slightly higher at pH values of 6.5 and 7.0 than at 5.5 or 6.0. Fat did not affect the survival of the organisms (Merkal and Crawford, 1979). In a study by Schulze-Robbecke and Buchholtz (Schulze-Robbecke and Buchholtz, 1992), D-values in suspension at 70ºC were calculated for *M. avium, M. intracellulare, M. marinum, M. chelonae, M. fortuitum* and *M. scrofulaceum.* The D-values did not exceed 6 seconds for any of the strains tested.

The Panel concludes that although it is difficult to predict exactly how the FSPM method will affect Mycobacterium *sp. without proper experimental data, the Panel believes that the FSPM method will inactivate* Mycobacterium *sp. in category 2 and 3 material of fish origin.*

Vibrio

Bacteria belonging to the genus *Vibrio* are Gram-negative, oxidase-positive, non spore forming and are typically found as slightly curved rods. The species within the genus *Vibrio* are indigenous and widespread in aquatic habitats at different salinities. These bacteria are common in marine and estuarine environments, and on the surfaces of marine plants and animals (Baumann *et al.,* 1984). They also occur in the intestinal content of marine animals (Sakata, 1990). Furthermore some *Vibrio* species are found in freshwater (West, 1989). More than twenty *Vibrio*-species have been described as able to cause disease in animals, while twelve species have so far been reported to be pathogenic to humans (West, 1989). Important fish and shellfish pathogenic vibrios are *Vibrio anguillarum* (currently *Listonella anguillarum*)*, Vibrio salmonicida*, *Vibrio splendidus* and *Vibrio viscosus (*currently *Moritella viscosa)* (Lillehaug *et al*., 2003). Of the human pathogenic species, *V. cholerae, V. vulnificus* and *V. parahaemolyticus* are predominant (Adams and Moss, 2008).

As the vibrios are adapted to the marine environment, they favour slightly alkaline conditions as found in seawater, with its pH of approximately 8. Vibrios are not considered to be robust bacteria, and are thus sensitive to heating, freezing, drying as well as low pH (Bylund *et al*., 1993; Oliver and Kaper, 2001). The poor ability of vibrios to survive at low pH, is reflected by the high infective dose of *V. cholerae* in humans, which may reach 10^8 to 10^{10} cells. This is due to a rapid bacterial decimation in the acidic environment found in the stomach.

The Panel concludes that considering the lack of spore formation and the limited survival of vibrios at low pH and increased temperature, the FSPM method will inactivate vibrios in category 2 and 3 material of fish origin.

Other non-sporeforming bacteria

Also other non-sporeforming bacteria like *Lactococcus garvieae, Aeromonas* sp. and *Franciscella* may be associated with category 2 and 3 by-products of fish origin. The panel has not made a thorough assessment concerning these species. However, like the other bacteria assessed above, non-sporeforming bacteria are generally sensitive to heat.

The Panel concludes that the FSPM method will inactivate all relevant, non-sporeforming bacteria in category 2 and 3 material of fish origin.

Sporeforming bacteria

Clostridium botulinum

Clostridium botulinum is a sporeforming Gram-positive obligate anaerobic bacterium. Most strains are toxin-producers and *C. botulinum* can cause botulism, which may be fatal (Lund and Peck, 2000). The strains of *C. botulinum* are often divided into four groups on the basis of physiological characteristics. Group I and II are referred to as proteolytic and non-proteolytic strains, respectively. Group I strains may produce toxins of type A, B and F, Group II: toxins B, E and F, Group III: toxins C and D, and Group IV: toxin G. Human botulism is almost always caused by toxins of types A, B, and E (Lund and Peck, 2000). Botulism also occurs in many types of animals, and is usually caused by toxins C and D. Outbreaks in birds caused by strains producing toxin E has been reported, due to the consumption of toxic fish (Lund and Peck, 2000).

C. botulinum is ubiquitous in nature and spores are naturally present in soil and waters. The main type found in marine sediments produce type E toxin (Johannsen, 1963; Lund and Peck, 2000). Studies from the 1960-ties showed that *C. botulinum* was frequently found in the Skagerak/Kategat area, probably originating from the Baltic Sea. Lower incidence was found in the North Sea and along the Western coast of Norway (Johannsen, 1963; Cann *et al*., 1965).

Fish is one of the main types of food implicated in human botulism. Botulism in birds, due to the consumption of toxic fish, has also been reported (Lund and Peck, 2000). The incidence of *C. botulinum* in freshly caught fish may be very low in many areas, but in some areas the reported incidence can be high. High incidences are reported in fish from the Swedish sound (100 %), Danish trout ponds (5-100 %) and in the Southern Baltic Sea (95 %). For herring in the Norwegian Sea and the Viking bank the incidences are reported to be 65 and 44 %, respectively. The majority of strains detected in fish were type E toxin producers (Lund and Peck, 2000; Lindström *et al.*, 2006). The number of *C. botulinum* in freshly caught fish is usually very low (<0.1/gram). The highest recorded number of *C. botulinum* type E spores on fresh fish is 5.3/gram, found in Danish farmed trout. The use of wet fish as feed was suggested as one of the reasons for this high number (Huss *et al.*, 1974). *C. botulinum* may proliferate to high numbers in dead fish (Eklund *et al.*, 1984; Gram and Huss, 2000). Botulism has been reported among freshwater reared salmonids in earth ponds and asphalt lined ponds, caused by cannibalism on dead fish (Huss and Eskildsen, 1974; Eklund *et al.*, 1984). Data on

the concentration of *C. botulinum* in dead fish from marine aquaculture systems are not available.

Data from a Norwegian factory showed that 12 out of 30 samples of raw fish silage analysed in 2006-2008 was positive for "sulphite reducing clostridia" (excluding *C. perfringens*). The concentrations varied from 60-150 000 pr. gram (Nofima, 2009b). The term "sulphite reducing clostridia" includes *C. botulinum*, but it is not known which species of *Clostridium* dominating in these samples.

The *C. botulinum* type E is reported to grow as low as at 3.3 °C, however the growth rate will increase at higher temperatures. For fish inoculated with 10^2 spores of a toxin E producer per gram, the time to toxin formation, at 4, 8 and 12 °C, were 18-46, 6-9 and 3-6 days, respectively (Lund and Peck, 2000).

The ability of *C. botulinum* to grow at acidic pH varies between strains, however no strains are able to grow and produce toxins at pH <4.2 (Lund and Peck, 2000). Spores of *C. botulinum* are reported to survive at acidic conditions. No reduction in viable count was observed for 56 days at pH 4.5-5.1 (Lund *et al*., 1985). In another study, spores of *C. botulinum* type A survived at pH 4.2 for 180 days at 4, 22 and 32 °C. For type B, the spores survived at pH 4.2 at 4 °C, however there was a 30% reduction in the number of spores after 180 days at 22 and 32 °C (Odlaug and Pflug, 1977).

The heat resistance of *C. botulinum* varies between strains. In general the strains can be divided in the heat-resistant proteolytic strains and the less resistant non-proteolytic strains. For the non-proteolytic strains $D_{82.2°C}$ of spores are reported in the range 0.4-32.1 min in phosphate buffer, pH 7. In whitefish chubs, tuna in oil and cod homogenate, $D_{82.2°C}$ of spores is reported to be 2.2, 6.6 and 8.7 min, respectively. The z-values are reported in the range 5.6-10 °C (Lund and Peck, 2000). $D_{85°C}$ values for type E spores in rainbow trout and crawfish has been reported as 2.0 and 6.7-8.8 min, respectively (Lindström *et al*., 2006). Guidelines for control of *C. botulinum* in fish recommend 90 °C for 10 min, in combination with chill storage (Huss and Gram, 2003). For the proteolytic strains, a $D_{121°C}$ of 0.2 min and a z-value of 10 °C are often used as the maximum heat resistance, calculated for phosphate buffer, pH 7 (Lund and Peck, 2000).

The presence of lytic enzymes in fish is reported to increase the heat resistance. Spores of *C. botulinum* have been described to be less heat resistant at acid pH. In one study in buffer, $D_{112.8°C}$ -value was decreased by 50 % at pH 5.0 compared to pH 7.0 (Hutton *et al*., 1991). In another study $D_{80°C}$ and $D_{90°C}$ was decreased by 42 and 35%, respectively, at pH 5.0 compared to pH 6.0 (Jujena *et al*., 1995). There is lack of data describing the effect on heat resistance after pre-exposure to acidic pH.

The type E toxin is sensitive to heat, however it tolerates more heat at acidic conditions (pH 4-5). For safe inactivation of the toxin, heat treatment at 79 °C for 20 min or 85 °C at 5 min is recommended (Huss and Gram, 2003).

The Panel concludes that after acidification to pH ≤4, C. botulinum *will not grow but most probably the number of spores will be stable until heat treatment. Spores of the proteolytic type will most probably be little affected by the FSPM method. However, it is reported that most strains of fish origin are non-proteolytic type E toxin producers that are less heat resistant. There are little data available to indicate effects on heat treatment in fish by lytic*

enzymes, low pH and fat, so the quantitative reduction of spore numbers by the FSPM method is very difficult to evaluate. The FSPM method will most probably lead to a reduction of non-proteolytic strains in the range of 3-10 log. For category 2 and 2 material from fish cultivated in marine waters in net cages where dead fish are removed on a daily basis and for category 3 material from fish cultivated in earth ponds, the levels of C. botulinum *will be so low that the FSPM method will inactivate* C. botulinum*. For Category 2 material from fish cultivated in earth ponds where dead fish are not removed on a daily basis, the concentration of* C. botulinum *may reach concentrations so high that the possibility of spores of* C. botulinum *type E surviving heat treatment cannot be ruled out. Furthermore the spores surviving heat treatment will not germinate as long a pH ≤4. Preformed toxins of type E will be inactivated by the FSPM method.*

Clostridium perfringens

Clostridium perfringens is a spore-forming Gram-positive obligate anaerobic bacterium that can produce toxins. *C. perfringens* is a common cause of gastroenteritis. *C. perfringens* can be found in marine environments and in fish, often as a result of sewage contamination. Fish is not commonly involved in foodborne outbreaks by *C. perfringens* (Labbè, 2000). The significance and importance of feed contamination by *C. perfringens* is not clear, however feed contaminated by *C. perfringens* has been involved in outbreaks in fowls (EFSA, 2008). Legislation commonly set limits for *C. perfringens* in by-products, where *C. perfringens* is considered as an indicator for heat-resistant organisms.

In Norway, a method for treatment of by-products from aquaculture at 85 °C for 25 min has been approved since 1994. In a testing period leading to the approval of the method, a total of 4 out of 15 samples were positive for *C. perfringens* for one of the factories using the method while for the two other factories no samples contained *C. perfringens* (Torgersen, 1994). During the last 10 years the method has been in use in a total of three factories. In one of the factories, one out of 215 samples of protein concentrate was positive for *C. perfringens*, while in the two other factories there were no positive samples (data enclosed with the Application). Documentation of the exact time/temperature combinations used in the factories was not available.

Toxin producing strains are more resistant to heat than non-producers. $D_{90°C}$ values are reported in the range 5.5-120.6 min (Orsburn *et al*., 2008). In a study where the processing conditions was similar as for the FSPM method (fish silage, 85 °C, pH ≤4, 25 min), the reduction of spores of *C. perfringens* was 2-3 log (Nofima, 2009 b). Data from a Norwegian factory showed that the concentration of *C. perfringens* in raw fish silage was <10 pr. gram in all 30 samples analysed in 2006-2008 (Nofima, 2009 b).

The Panel concludes that the FSPM method will inactivate C. perfringens *in category 2 and 3 material of fish origin.*

Moulds

Moulds are a diverse group of microorganisms that are ubiquitous in nature and exist as saprophytes or pathogens (Pitt and Hocking, 1999). Moulds and mycotoxins are among the common contaminants that reduce nutritional value, quality and safety of both feed raw materials and final products by affecting animal health, productivity and food safety.

The true fungi are eukaryotic organisms belonging to the kingdom *Eumycota.* Important fish fungal pathogens are *Aphanomyces invadans*, *Aphanomyces astaci* and *Ichthyophonus hoferi.*

Optimum pH for mould growth is slightly acidic, but most moulds are capable to grow at pH 2.5 – 9.5, some even down to pH 1.5. Temperature optimum for growth and toxin production is ca 25 °C for most moulds (mesophilic). However some moulds, e.g. some Aspergilli, are termophilic and are able to grow and produce mycotoxins up to at least 58 °C, while others, e.g. certain *Penicillium* species, are regarded psykrophilic and are able to grow and produce mycotoxins down to –7 °C.

Most of the fungi affecting fish are strictly aquatic and cannot survive outside an aqueous environment. Their infectivity is rapidly reduced to negligible levels at temperatures above 40 °C. Therefore treatments already described to reduce the infective tires of viruses, bacteria and parasites, are also likely to reduce the infectivity of pathogenic fungi to negligible levels (EC, 2003).

The Panel concludes that the FSPM method will inactivate fungi in category 2 and 3 material of fish origin.

Mycotoxins

Moulds cause quality and safety problems indirectly by producing mycotoxins as their metabolic by-products. Mycotoxins are fungal secondary metabolites that in small concentrations are toxic to vertebrates when introduced via a natural route (Pitt and Hocking, 1999). Over 300 identified mycotoxins are known to induce signs of toxicity in animals and their number is increasing.

Mycotoxins may be present in fish feed, although limited information is available compared to human feed. Aflatoxins ingested via feed cause pale gills, impaired blood clotting, anaemia, poor growth rates or lack of weight gain, liver tumours, and in some cases mortality (Santacroce *et al*., 2008). Channel catfish (*Ictalurus punctatus*) fed ochratoxin A contaminated feed showed reduced growth and lesions in hepatopancreatic tissue (Manning *et al*., 2003) while other mycotoxins are known to cause various health problems and reduced productivity of fish (Encarnacao, 2006; Santacroce *et al*., 2008; Tacon and Metian, 2008).

Due to their low molecular weight and chemical structure, mycotoxins are very stable. Trichothecenes like deoxynivalenol (DON) and T-2 toxin are stable at 120 °C and moderately or partially stable at 180°C or 210 °C. Fumonisins are quite heat stable and for this reason the toxin content can not be reduced significantly at processes below 150 °C. Additionally, the effect of heat treatment often depends on exposure time and pH. Zearalenone is also very stable and moreover the content of this toxin does not decrease at high temperatures. Ochratoxins are fairly stable and can only partially degrade during cooking and fermentation processes (Jouany, 2007).

For some of the mycotoxins, possible accumulation in fish tissues should be investigated of particular importance for the end user (humans). A range of mycotoxins will potentially elicit acute adverse effects in fish, as in other animals, but so far, knowledge on toxicokinetics of main mycotoxins in fish is scarce and corresponding toxicity data fragmentary. The Norwegian Scientific Committee for Food Safety concluded in their opinion *Criteria for safe use of plant ingredients in diets for aquacultured fish* (VKM, 2009): “Studies of dietary exposure to undesirable substances, e.g. pesticides and mycotoxins, and their toxic effects and toxicokinetics in fish are scarce”. There is no knowledge of potential effect of mycotoxins from fish on animal or human health. Thus there is no evidence that mycotoxins are more

associated with category 2 and 3 materials of fish origin than healthy fish slaughtered for human consumption.

The Panel concludes that the FSPM method will not inactivate mycotoxins, however it has not been shown that mycotoxins from fish may pose a hazard to animal or human health.

Saprolegnia

Saprolegnia spp. belongs to the class *Oomycetes*, which is a group of fungi-like pathogens in the kingdom *Straminiphila* (Dick, 2001). *Oomycetes* have their phylogenetic roots with the chromophyte algae (which includes the diatoms, chrysophytes and brown seaweeds) rather than with the main evolutionary line of chitin containing fungi (Beakes, 1989). *Saprolegnia* infection is traditionally known as "fungal infection" in fish, and is typical seen in the fresh water stage of salmonids. *Saprolegnia* infections are visible as white patches on the skin of the fish, or as "cotton wool" on fish eggs. The "fungal" patches may consist of one or more species of *Saprolegnia* (Pickering and Willoughby 1982). The disease was previously controlled by the use of malachite green, an organic dye which has proved very efficient in controlling all infectious stages of *Saprolegnia* spp. The use of malachite green has been banned in Norway and other parts of the world due to its potential toxicological effects. This has increased the incidence of *Saprolegnia* infections in the aquaculture all over the world, resulting in huge economic losses.

Saprolegnia (Sa.) diclina and *Sa. parasitica* were shown to germinate in strong acidic conditions by Kitancharoen (Kitancharoen *et al.,*1996). *S. diclina* isolates appeared to germinate at a pH condition as low as 3.5, whereas *Sa. parasitica* isolates could not germinate at below pH 3.8. *Sa. parasitica* isolates from visceral mycoses still showed good growth at 30°C. Also, *Sa. parasitica* isolates from visceral mycoses produced more abundant motile zoospores, and continued to do so for a longer period of time (28 d), than *Sa. parasitica* isolates from external saprolegniasis and *Sa. diclina* isolates.

The Panel concludes that the FSPM method will inactivate Saprolegnia in category 2 and 3 material of fish origin.

Parasites

Parasites are commonly found in or on fish, crustaceans, bivalves and other marine organisms. Relevant parasite species may belong to various groups including fungi, protozoans, myxosporidians, flukes, tapeworms, roundworms and arthropods. The vast majority of these parasites pose no direct threat to the animal or human health. However, according to Huss (Huss, 1995), more than 50 species of helminth parasites from fish or shellfish may cause disease in humans. The metazoan parasites most often associated with human disease after ingestion of under-processed seafood, may be coarsely classified into tapeworms, flukes and roundworms (Higashi, 1985). In Norwegian waters, the larvae of several species of roundworms (nematodes) belonging to the genera *Anisakis*, *Pseudoterranova, Contracaecum* and *Phocascaris* are presently the most important parasites regarding both the quality and consumer safety of fishery products. Nematode larvae commonly occur in virtually all marine fish species, and are normally found encapsulated in or around the visceral organs, but some may also penetrate into the fish flesh, sometimes lodging deeply within the fillets (Berland, 1961; Levsen *et al*., 2008). However, farmed fish such as Atlantic salmon, which are exclusively fed on dry or expanded pelleted feed, do

apparently not carry any nematode larvae in the flesh (Angot and Brasseur, 1993; Bristow and Berland, 1991; Lunestad, 2003; Marty, 2008). Although the main components of such dry fish feed originate from marine raw material, any possibly present nematode larvae are killed during the processing of the feed. However, if the absence of nematodes in the fish flesh also applies for other sea pen reared fish species such as Atlantic cod or halibut, has so far not been thoroughly investigated.

Parasites which live in, or migrate through, the gastrointestinal tract of their host(s) are *per se* adapted to low pH levels. Thus, any such parasite may survive under the pH conditions proposed in the current method (Solas *et al*., 2009).

However a temperature of 85 ºC will kill any parasites possibly found in farmed or wild living fish (Levsen *et al*., 2008). In the literature temperatures between 60 and 77ºC has been given as sufficient to destroy any known parasite if found in fish from temperate waters (Adams *et al*., 1999, Levsen *et al*., 2008).

The Panel concludes that the FSPM method will inactivate all known types of parasites in category 2 and 3 material of fish origin.

Antibiotic resistance genes

Antibiotic resistance among bacteria is a constant challenge in all situations where antibacterial agents are used, possibly compromising the therapeutic outcome of anti-infective treatments in man and animals. The spread of antibiotic resistance determinants by transfer of DNA among bacteria, by transformation, transduction or conjugation, are well described in the literature.

If DNA coding for antibiotic resistance are present among bacteria in fish for production of silage, it will be released when the bacteria disintegrate. If not inactivated, such DNA may be a potential source for transfer of resistance by transformation, *i.e.* uptake of free DNA. A prerequisite for transfer of such resistance is the presence of intact DNA. The stability of free DNA is known to be influenced by environmental conditions such as nuclease activity, temperature and pH (Bauer *et al*., 2003; Nielsen *et al*., 2007).

In the study by Bauer (Bauer *et al*., 2003), the authors identify pH and temperature as the main factors causing DNA degradation in food. In an experiment with tomato juice with a pH of 4.3 and kept at 65 °C, 99 % degradation was observed within 90 minutes. The authors conclude that DNA fragments of sufficient length to confer functional information is not expected to be found in acidified and heat treated food, and that the main factor for DNA degradation is the low pH. The effect of low pH is also shown in reports on the stability of DNA under the conditions found in the mammalian digestive system. Under these conditions the biological activity of free DNA is considered to be short lived (Duggan *et al*., 2003; Heritage, 2002).

The Panel concludes that free DNA will most probably not retain its functionality after treatment with the FSPM method, and such DNA would thereby not be able to transfer antibiotic resistance.

Prions

Prions are proteinous particles able to give infection in mammals, including humans. Infections by prions are collectively known as ”transmissible spongiform encephalopathy” or TSEs, and are histopathologically characterized by degenerative changes in the brain of the

infected individual. These infective proteins will maintain infectivity after heat treatment, irradiation and exposure towards disincentive agents. It is not likely that the FSPM method will inactivate prions.

Many species of fish are carnivorous and cannibalistic in the wild and hence have the potential to recycle TSEs if present in fish. Likewise the farming of many species is based on feed containing processed fish by-products (EC, 2003). Prions are present in fish (Oidtmann *et al.,* 2003), e.g. salmon (Gibbs and Bolis, 1997) and pufferfish (*Fugu rubripes*) (Suzuki *et al.,* 2002). The fish prions however, are different from mammalian proteins in key sites, and it has been considered most unlikely that transmission of possible reverted prions could occur between fish and mammals (Joly *et al.,* 2001, Rivera-Milla *et al.,* 2003). The limited transmission studies that are currently in progress have so far not provided evidence of TSE disease or infectivity replication in fish (EC, 2003). Thus there is no evidence that prions are more associated with category 2 and 3 materials of fish origin than healthy fish slaughtered for human consumption.

The panel concludes that the FSPM method will not inactivate potential prions in byproducts from fish, however it is not likely that prions from fish may pose a hazard to animal or human health.

CONCLUSION

Answer to the terms of reference
To assess how the described processing method will affect relevant microbiological risks for category 2 and 3 material of fish origin.

The Panel concludes that the FSPM method will inactivate non-sporeforming bacteria, *Clostridium perfringens*, moulds, *Saprolegnia*, parasites and viruses in category 2 and 3 material of fish origin.

For category 2 and 3 material from fish cultivated in marine waters in net cages where dead fish are removed on a daily basis, and for category 3 material for fish cultivated in earth ponds, the levels of *C. botulinum* will be so low that the FSPM method will inactivate *C. botulinum*

For category 2 material from fish cultivated in earth ponds where dead fish are not removed on a daily basis, the concentration of *C. botulinum* is reported to reach levels so that the possibility of spores of *C. botulinum* type E surviving heat treatment cannot be ruled out. The spores surviving heat treatment will not germinate as long a pH $\leq$4 is maintained. Preformed toxins of type E will be destroyed by the FSPM method.

The FSPM method will degrade DNA, thus genes potentially encoding antibiotic resistance will be inactivated.

The FSPM method will not inactivate mycotoxins, however it has not yet been shown that mycotoxins in fish may pose any hazard which demands particular treatment concerning animal or human health.

The FSPM method will not inactivate potential prions in byproducts from fish, however it is not likely that prions from fish may pose any hazard to animal or human health.

Data gaps

In general there is a lack of data on how reduction of microorganisms is affected by the combination of low pH and heat in specific materials.

There is limited knowledge available on mycotoxins in fish feed, and their potential impact on fish, animal and human health.

There is limited knowledge of prions associated with fish. Also their potential infectivity between different fish species or from fish to animals or humans has been little studied.

Data on the concentration of *C. botulinum* in dead fish from marine aquaculture systems are not available.

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