Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept

EU Integrated Project no 506359

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SEAFOODplus Publication Series
Report 3.4.2 - 2008
ISBN 978-87-7075-005-9

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A report from RTD area 3

'Seafood safety'

Project 3.4 BIOCOM

Results of biogenic amine concentrations and microflora in seafood causing histamine fish poisoning (HFP)

Deliverable D2 2005

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1. Introduction

The overall objective of the BIOOCOM-project is to provide data that will reduce the intake of biogenic amines from seafoods by European consumers and reduce the incidence of histamine fish poisoning (HFP). Histamine production in seafood is related to the histidine content of the muscle tissue since histamine is produced by bacterial decarboxylation of the amino acid histidine (Lehane and Olley 2000).

To reduce the formation of histamine and other biogenic amines in seafood the mechanisms leading to their formation must be understood. It is most important to determine the groups of microorganisms that produce the concentrations of histamine and other biogenic amines actually resulting in outbreaks and cases of HFP. Thousands of cases of HFP have been recorded worldwide and linked to intake of seafood with high concentrations of histamine (See Taylor 1986 and Emborg 2004: Table 1 in BIOCOM Deliverable D1 'Gaps in available information on biogenic amines in seafood'). It is generally accepted that HFP is caused by seafoods with more than 500-1000 mg/kg of histamine. Nevertheless, the concentration of histamine within a single fish can vary by orders of magnitude (See Emborg et al. 2005) and consequently samples of seafood that caused HFP have been shown subsequently to contain only low concentration of histamine. In relation to HFP several important issues remain incompletely understood including:

1. Which are the groups of microorganisms responsible for histamine formation in seafood that cause HFP?
2. Are high concentrations of biogenic amines like cadaverine, putrescine and tyramine stimulating the toxic effect of histamine?

It is well established that several species of mesophilic Enterobacteriaceae including *Morganella morganii, Raoultella planticola* (previously *Klebsiella pneumoniae*), *Raoultella ornitinolytica, Enterobacter aeroginosa* and *Proteus vulgaris* can produce large amounts of histamine in seafoods and model substrates at temperatures above 7-10°C (See Emborg 2004: BIOCOM Deliverable D1 'Gaps in available information on biogenic amines in seafood'). Probably as a result of observations like these it has been accepted by many that the formation of high concentration of histamine in seafood and HFP is related to products that have been exposed to high temperatures (See e.g. (FDA 2001) and (Kim et al. 2004)). Despite thousands of reported cases on HFP the number of outbreaks where the bacteria responsible for histamine formation in the implicated seafoods were identified remains extremely low. In fact, we have been able to identify only six documented HFP outbreaks between 1955 and 2003 where the bacteria responsible for histamine formation was identified (Table 1). It needs to be noted that out of these six HFP outbreaks the two latest reported by (Kanki et al. 2004) and (Emborg et al. 2005) were caused by psychrotolerant bacteria. These psychrotolerant bacteria were able to grow and produced more than 1000 ppm of histamine at 2-4°C. It also should be noted that the concentration of biogenic amines other than histamine have not been reported in relation to documented HFP outbreaks prior to 2003 (Table 1).

To mitigate the risk of HFP it is important to know if the bacteria responsible for histamine and biogenic amine formation are psychrotolerant and able to grow and produce histamine at 2-4°C or whether they are mesophilic and only produce

significant concentration of histamine in seafood when kept at high temperatures e.g. above 7-10°C. Consequently the BIOCOM project was designed to evaluate the importance of both psychrotolerant and mesophilic bacteria and to take into account not only the concentration of histamine but also the concentration of other biogenic amines. To meet its overall objective the BIOCOM project is divided into four closely related work-packages. The first work-package concerns '*Outbreaks and cases of histamine fish poisoning*'. This report describes the results obtained within this work-package during the first 18 months of the BIOCOM project. Main findings are reported just below and summarized in Table 2 whereas a detailed description of the studied outbreaks and cases are provided in section 3.

2. Summary and conclusions

During the first 18 project-months 12 outbreaks or cases of HFP occurred in Denmark and 53 persons became ill (i.e. 53 cases). The BIOCOM project obtained sample material from all of the 12 implicated seafoods and product characteristics including the concentration of histamine and other biogenic amines were determined. In 9 of the 12 implicated products histamine concentrations above 500-1000 mg/kg were detected. However, concentrations of other biogenic amines were much lover than the concentrations of histamine. Thus we have not been able to support the hypothesis that HFP should be cause by intake of histamine in combination with high concentration of other biogenic amines. In fact, our results indicate that HFP can be caused by seafood with high concentrations of histamine even when concentration of other biogenic amine are below 200 ppm (HFP no. 2, 8 9 and 11) (Table 2).

From 4 of the 12 implicated products the BIOCOM project has been able to isolate bacteria that form high concentration of histamine and therefore must have been responsible for histamine formation in the implicated products. The bacteria responsible for histamine formation were psychrotolerant *Photobacterium phosphoreum* (HFP no. 1), psychrotolerant *Morganella morganii*-like bacteria (HFP no. 2), mesophilic *Morganella morganii* (HFP no. 6) and a bacteria from HFP no. 12 that produced more than 8,000 mg/kg of histamine but has not yet been identified (Table 2).

The BIOCOM project has very significantly increased the existing information about bacteria responsible for histamine formation in seafood implicated in HFP (Table 1, Table 2). Importantly we showed psychrotolerant bacteria were responsible for histamine formation in two products implicated in HFP whereas we found only one implicated product where histamine formation was due to the well known mesophilic histamine producing *Morganella morganii.*

We conclude in agreement with Kanki et al. (2004) and Emborg et al. (2005) that histamine formation by psychrotolerant bacteria previously has been and overlooked risk factor in relation to HFP. To comply with its objectives the BIOCOM project will continue to focus on psychrotolerant bacteria and their importance for HFP.

Table 1. Outbreaks and cases of histamine fish poisoning (HFP) that occurred prior to the BIOCOM-project and in relation to which the microorganisms responsible for histamine formation in the products have been identified.

Year	Country	Products	Cases	Biogenic amines, mg/kg				Bacteria responsible for histamine formation	References
				Hist[a]	Cad	Put	Tyr		
1955	Japan	Fresh tuna	50	1,190	NR[b]	NR	NR	*Morganella morganii*	(Kawabata et al. 1956)
1965	Japan	Fresh tuna	?	?	?	?	?	*Morganella morganii*	(Sakabe 1973)
1967	Czechoslovakia	Fresh tuna	?	120-3,120	NR	NR	NR	*Hafnia sp.*	(Havelka 1967) (Ferencik 1970)
1977	USA	Fresh tuna	15	1,600-9,190	NR	NR	NR	*Raoultella planticola*	(Lerke et al. 1978)
2002	Japan	Dried sardines	1	3,000	NR	NR	NR	*Photobacterium phosphoreum*	(Kanki et al. 2004)
2003	Denmark	Tuna in chili sauce	8	7,100-9,100	27-54	14-16	53-70	*M. morganii-like; P phosphoreum*	(Emborg et al. 2005)

[a] Histamine (Hist.), cadaverine (Cad), putrescine (Put) and tyramine (Tyr).
[b] Not reported (NR).

Table 2. Outbreaks and cases of histamine fish poisoning (HFP) studied within the BIOCOM-project during project-months 0-18.

Year	HFP	Products	Cases	Biogenic amines, mg/kg				Bacteria responsible for histamine formation
				Hist[a]	Cad	Put	Tyr	
2004	1	Cold-smoked tuna	2	4,550	213	20	140	*Photobacterium phosphoreum*
2004	2	Cold-smoked tuna	1	1,973	132	6	88	*M. morganii*-like bacteria (psychtotolerant)
2004	3	Tuna sandwich, canned tuna	2	< 5	< 5	< 5	14	No strong histamine producing isolates
2004	4	Escolar	7	< 5	< 5	< 5	< 5	No isolates
2004	5	Cooked escolar,	4	4,090	257	< 5	17	No isolates
2004	6	Tuna heated in flexible film	8	6,432	286	40	< 5	*M. morganii* (mesophilic)
2004	7	Tuna (Frozen and cooked)	1	< 5	< 5	< 5	< 5	No isolates
2004	8	Cold-smoked tuna	10	914	68	< 5	23	No isolates
2004	9	Swordfish in saffron sauce	4	1,348	153	< 5	< 5	No isolates
2005	10	Escolar, marinated	7	5,810	321	< 5	< 5	No strong histamine producing isolates
2005	11	Tuna	2	96-1,738	< 5 - 112	< 5	< 5	No strong histamine producing isolates
2005	12	Smoked escolar	5	1,705	224	< 5	< 5	One strong histamine producing isolate

[a] Histamine (Hist.), cadaverine (Cad), putrescine (Put) and tyramine (Tyr).

3. Biogenic amine concentrations and microflora in seafood causing histamine fish poisoning (HFP) BIOCOM project months 0-18

Samples of seafoods involved in outbreaks have been collected and analysed by chemical and microbiological methods. In addition a questionnaire has been used to collect information about (i) the type of product and how it was purchased and prepared prior to consumption, (ii) amount of the seafood consumed as well as simultaneous consumption of other foods that may cause histamine fish poisoning, (iii) number of people consuming the seafood and number of people that became ill as well as (iv) symptoms and medical treatment.

Histamine fish poisoning outbreak 1 (Cold-smoked tuna): Ultimo January 2004 two persons got ill after eating cold-smoked tuna. Symptoms including nausea, rash and a tingling sensation in the mouth were observed 45 minutes after consumption and lasted about 12 hours. In the remaining (not eaten) parts of the implicated tuna product the concentration of histamine was 4,500-4,600 mg/kg. The concentration of other biogenic amines were: tryptamine (<5 mg/kg), phenylethylamine (51-57 mg/kg), putrescine (19-20 mg/kg), cadaverine (210-215 mg/kg), serotonine (<5 mg/kg), tyramine (140 mg/kg), spermidine (<5 mg/kg) and spermine (< 5 - 14 mg/kg). The product had pH 6.1 and contained 28% dry matter but only 0.9% NaCl. Analysis of free amino acids showed a concentration of free histidine of 7,156±317 mg/kg. Thus very significant concentrations of histamine can potentially be produced in this product. Microbiologically the samples were analysed for Aerobic plate count (29,700,000 cfu/g), "Luminous bacteria" (28,000 cfu/g), Enterobacteriaceae (280 cfu/g) and Lactic acid bacteria (6,300,000 cfu/g). 21 isolates from outbreak 1 were isolated and identified to the species level (five isolates of *Photobacterium phosphoreum* and two of *Pseudomonas fluorescens*) or to the genus level (seven isolates of *Lactobacillus/Weisella*, one of *Carnobacterium* and four of *Brochothrix*). Two isolates remained unidentified lactic acid bacteria. Eight of these isolates have been screened for their ability to form histamine and other biogenic amines in a liquid laboratory medium at 10°C. Only the *P. phosphoreum* isolates produced histamine. These isolates were able to produce large amounts of histamine (3,800 mg/l) and it is concluded that *P. phosphoreum* was responsible for producing the 4,500-4,600 mg/kg of histamine observed in the cold-smoked tuna.

Histamine fish poisoning outbreak/case 2 (Cold-smoked tuna): Medio April 2004 one person got ill after eating cold-smoked tuna. Symptoms including rash, increased pulse and hyperventilation started short after consumption and lasted about two hours. In the remaining part of the implicated product the concentration of histamine was 1,975 mg/kg. Concentrations of other biogenic amines were tryptamine (<5 mg/kg), phenylethylamine (<5 mg/kg), putrescine (6 mg/kg), cadaverine (130 mg/kg), serotonine (<5 mg/kg), tyramine (90 mg/kg), spermedine (<5 mg/kg) and spermine (<5 mg/kg). The product had pH 5.9 and contained 27% dry matter and 1.6% NaCl. Analysis of free amino acids revealed an unusually high concentration of arginine (~11,000 mg/kg) but the expected high concentrations of histidine (~16,000 mg/kg). Microbiologically the sample was analysed for Aerobic plate count (150,000,000 cfu/g), "Luminous bacteria" (not detected) and Enterobacteriaceae (631,000 cfu/g). 14 bacterial isolates from the dominating microflora of the cold-smoked tuna were isolated and identified to the species level (five isolates of psychrotolerant *Morganella*

morganii-like bacteria) or to the genus level (*Aeromonas, Carnobacterium, Providencia* and *Pseudomonas*). Nine of these isolates have been screened for their ability to form histamine and other biogenic amines in a liquid laboratory medium at 10°C. Only the *M. morganii*-like isolates produced large amounts of histamine and it is concluded that the *M. morganii*-like bacteria was responsible for producing the ~ 2,000 mg/kg of histamine observed in the cold-smoked tuna. Interestingly the *M. morganii*-like bacteria responsible for the histamine formation in cold-smoked tuna implicated in outbreak 2 were psychrotolerant and had the ability to grow at 2°C but not at 37°C. Thus these bacteria differ markedly from mesophilic *M. morganii* previously reported to produce histamine in seafood.

Histamine fish poisoning outbreak 3 (Tuna sandwich/canned tuna): Ultimo May 2004 two persons got ill after eating tuna sandwich prepared using canned tuna. Symptoms including nausea, rash, diarrhoea and a tingling sensation in the mouth appeared ca. 15 minutes after consumption and lasted 5-6 hours. Canned tuna was obtained from the same shop but it is uncertain if tuna was obtained from the same can that caused the outbreak. The concentration of histamine was <5 mg/kg. Concentrations of other biogenic amines were: tryptamine (<5 mg/kg), phenylethylamine (19 mg/kg), putrescine (<5 mg/kg), cadaverine (<5 mg/kg), serotonine (<5 mg/kg), tyramine (14 mg/kg), spermidine (<5 mg/kg) and spermine (<5 mg/kg), The tuna sandwich filling had pH 5.3 and contained 0.9 % NaCl and 37% dry matter. Microbiologically the sample was analysed for Aerobic plate count (33,000,000 cfu/g), “Luminous bacteria” (not detected), Enterobacteriaceae (250,000 cfu/g) and Lactic acid bacteria (50,000,000 cfu/g). 14 isolates from aerobic plate counts were characterized and 7 representative isolates were screened for their ability to produce histamine and other biogenic amines in a liquid laboratory medium at 10°C. Of 9 Gram-positive bacteria, 5 were tested for histamine formation and none produced histamine whereas the remaining Gram-negative (*Aeromonas*-like) isolates were moderately strong histamine producers. As the tuna sandwich filling obtained from the restaurant did not contain a detectable concentration of histamine the two cases of HFP will remained poorly understood. The tuna sandwich filling contained 50 million bacteria pr. gram but these most likely originated from other ingredients than the canned tuna.

Histamine fish poisoning outbreak 4 and 5 (Escolar): Both outbreaks were due to Escolar (*Lepidocybium flavobrunneum*). This fish species is often referred to a butterfish although this can be misleading. In early July 2004, seven people got ill after eating escolar in a canteen (outbreak 4). Unconsumed material from the implicated product was not obtained but subsequently ten fillets of escolar from the same batch/seafood company was analysed. However, no histamine was detected in any of these fillets and further analyses were not carried out. To evaluate the potential for histamine formation the concentration of free amino acids were determined. The escolar samples contained > 9,000 mg/kg of free histidine and > 2,500 mg/kg of free arginine. Thus, high concentration of histamine, agmatine, putrescine, spermidine and spermine may be formed in escolar.

Outbreak 5 in late July 2004 concerned oven-fried escolar consumed by ~50 people in the canteen of a company. Four persons got ill. Symptoms including nausea, vomiting and abdominal pain appeared ca. 5 minutes after consumption. 4,090 mg/kg of histamine was determined in the oven-fried escolar that actually caused the outbreak. The concentrations of other biogenic amines were: tryptamine

(<5 mg/kg), phenylethylamine (<5 mg/kg), putrescine (<5 mg/kg), cadaverine (260 mg/kg), serotonine (<5 mg/kg), tyramine (17 mg/kg), spermidine (<5 mg/kg) and spermine (17 mg/kg). As expected, due to the heat treatment, no bacteria were detected in the fried escolar. Analysis of three additional frozen and non fried escolar fillets showed histamine concentrations of < 5 – 128 mg/kg. In agreement with the low histamine concentration in these samples the aerobic plate counts were only 27,000-140,000 cfu/g and it was decided not to identify and further characterize the dominating micro-flora from these samples. The fried escolar implicated in outbreak 5 had pH of 6.26 and contained 48.3% dry matter and 0.9% water phase salt.

Histamine fish poisoning outbreak 6 (Heat treated tuna): Primo September 2004 at least eight people got ill after eating tuna. Symptoms included diarrhoea (8 persons), rash (3 persons) and nausea (2 persons). The tuna product was packed in a flexible film and heat-treated. In the remaining (not eaten) part of the tuna product the concentration of histamine was 6,400 mg/kg. Besides histamine, the concentration of other biogenic amines were: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrescine (40 mg/kg), cadaverine (290 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). The product had pH 6.3 and contained 0.7% NaCl and 25% dry matter. Microbiologically the samples were analysed for Aerobic plate count (2,5000,000 cfu/g), “Luminous bacteria” (<100cfu/g), Enterobacteriaceae (380,000 cfu/g) and Lactic acid bacteria (800 cfu/g). Ten colonies from the dominating micro flora and 7 colonies of Enterobacteriaceae were isolated and pure cultured. Of the 17 isolates 11 were identified at mesophilic *Morganella morganii* whereas 6 were *Enterobacter cloacae.* Histamine and biogenic amine formation were evaluated for three *M. morganii* isolates and three *E. cloacae. M. morganii* formed 8,480±350 mg/l histamine and 11.4±2 mg/kg of phenylethylamine whereas *E. cloacae* from < 5 to 180 mg/l of histamine. Formation of other biogenic amines was < 5 mg/kg. Thus, mesophilic *M. morganii* most likely has been responsible for formation of the 6,400 mg/kg of histamine in the implicated tuna product. The mesophilic *M. morganii* isolates were unable to grow at 2 °C and 4 °C and the product must have been stored at higher temperatures where *M. morganii* have grown to high concentrations and formed histamine in the product. The tuna product was heat-treated but the flexible packaging film may not have been sealed appropriately so that the product became contaminated with *M. morganii* after the heat treatment.

Histamine fish poisoning outbreak 7 (Frozen tuna): Primo September 2004 one person got ill after eating a tuna steak that had been purchased in a supermarket in the frozen state and cooked prior to consumption. Symptoms included severe rash, increased pulse, headache and respiratory problems. The person received antihistamine treatment. The tuna steak was not available for further analysis but two tuna steaks form the same supermarket/processor were analysed. The concentration of histamine and other biogenic amines was below < 5 mg/kg, pH was 5.8, dry matter 27% and the concentration of NaCl < 0.1%. The two samples were analysed for Aerobic plate count (approx. 350,000 cfu/g), “Luminous bacteria” (<100cfu/g), Enterobacteriaceae (approx. 200 cfu/g) and Lactic acid bacteria (approx. 1,400 cfu/g). Thus, the outbreak will remain poorly understood.

Histamine fish poisoning outbreak 8 (Cold-smoked tuna): Ultimo September 2004, 65 persons consumed cold-smoked tuna in a canteen and ten persons got ill. Symptoms

included vomiting and diarrhoea. In a frozen sample of the implicated cold-smoked tuna the concentration of histamine was 914 mg/kg. The concentration of other biogenic amines were: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrescine (< 5 mg/kg), cadaverine (68 mg/kg), serotonine (< 5 mg/kg), tyramine (23 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). The sample contained 3.6% NaCl. Aerobic plate count were only 4,500 cfu/g and the concentration of "Luminous bacteria" and Enterobacteriaceae were <100 cfu/g. Due to the very low concentration of bacteria and because the implicated sample of cold-smoked tuna had been frozen bacteria were not isolated for further identification and characterization. Thus, the bacteria responsible for production of the 914 mg/kg of histamine will not be identified.

Histamine fish poisoning outbreak 9 (Fried swordfish in saffron sauce): Primo November 2004 at least 4 people got ill after eating fried swordfish in saffron sauce. In remaining parts of the product the concentration of histamine was 280 mg/kg and 2,400 mg/kg. Besides histamine the samples contained tryptamine (< 5 mg/kg), phenylethylamine (11 mg/kg), putrecine (< 5 mg/kg), cadaverine (150 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). The product had pH 6.5 and contained 31% dry matter. As the samples had been fried/heated no microbiologically analyses were carried out.

Histamine fish poisoning outbreak 10 (Escolar/butterfish, frozen and marinated, two samples): Ultimo January 2005 at least 7 people got ill after eating escolar/butterfish. In the remaining parts of the product the content of histamine was estimated to 5,800 and 4,700 mg/kg. Besides being analyzed for histamine, the samples was analyzed for the content of other biogenic amines: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrecine (< 5 mg/kg), cadaverine (320 / 260 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). The fish flesh had a pH of 6.5 and 6.4 and a content of NaCl was 0.07% and 0.03%, respectively. The content of dry matter was 48.3±6.6% and 44.0±0.7%. Microbiologically the samples were analysed for Aerobic plate count (2,200,000 / 78,000 cfu/g), "Luminous bacteria" (<100 cfu/g), Enterobacteriaceae (11,000 / 1,500 cfu/g) and Lactic acid bacteria (570,000 / 35,000 cfu/g). 12 bacterial colonies were isolated and all were Gram positive. 5 isolates were tentatively identified as *Brochothrix* spp due to growth on STAA and production of catalase. The remaining 7 isolates were tentatively identified as *Carnobacterium* spp due to absence of growth on acetate agar and a final pH in La-broth above 4.15. *Brochothrix* spp and *Carnobacterium* spp do not produce histamine and formation of histamine in the implicated product most likely will remain unexplained.

Histamine fish poisoning outbreak 11 (Fresh red tuna, two samples): Medio February 2005 two persons got ill after eating tuna. In the remaining parts of the tuna the content of histamine was estimated to 96 and 1,700 mg/kg. Besides being analysed for histamine, the samples were analysed for the content of other biogenic amines: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrecine (< 5 mg/kg), cadaverine (< 5 / 110 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermedine (< 5 mg/kg) and spermine (< 5 mg/kg). The tuna parts had a pH of 6.1 and 6.3, a NaCl content of 0.03% and 0.07% and a dry matter content of 25 and 27%. Microbiologically the two samples were analysed for Aerobic plate count (200,000,000 / 120,000,000 cfu/g), "Luminous bacteria"(<100 cfu/g),

Enterobacteriaceae (2,700,000 / 830,000 cfu/g) and Lactic acid bacteria (150,000,000 / 58,000,000 cfu/g). Isolates from the sample with low histamine content were identified as *Brochothrix* spp. (5 isolates), Lactic acid bacteria (2 isolates) and *Shewanella* sp. (3 isolates). Isolates from the sample with high histamine content were identified as *Brochothrix* spp (9 isolates), Lactic acid bacteria (1 isolates), *Pseudomonas* sp. (1 isolate), *Providencia* sp. (2 isolates) and *Shewanella* sp. (1 isolate). *Pseudomonas* sp., *Providencia* sp. and *Shewanella* sp. produced < 5 mg/kg of histamine in broth at 10°C and histamine formation in relation to this outbreak could not be explained.

Histamine fish poisoning outbreak 12 (Smoked escloar/butterfish, two samples (remaining part from outbreak and an unopened package)): Ultimo February 2005 five persons got ill after eating smoked escolar/butterfish. In the remaining part of fish from the outbreak the concentration of histamine was 1705 mg/kg. Besides being analysed for histamine, the sample was also analysed for the content of other biogenic amines: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrecine (< 5 mg/kg), cadaverine (220 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). The fish flesh had pH 5.7, a NaCl content of 5.8% and a dry matter content of 47%. The sample from the unopened package had a histamine content of 2,600 mg/kg. The concentration of the other biogenic amines were: tryptamine (< 5 mg/kg), phenylethylamine (< 5 mg/kg), putrecine (< 5 mg/kg), cadaverine (370 mg/kg), serotonine (< 5 mg/kg), tyramine (< 5 mg/kg), spermidine (< 5 mg/kg) and spermine (< 5 mg/kg). Furthermore, the sample had a pH of 5.8, a NaCl content of 1.9 % and a dry matter content of 41%. Microbiologically the sample from the outbreak were analysed for Aerobic plate count (180,000 cfu/g), “Luminous bacteria” (<100 cfu/g), Enterobacteriaceae (130 cfu/g) and Lactic acid bacteria (150,000 cfu/g). Microbiologically a sample from unopened package was analysed for Aerobic plate count (2,500,000 cfu/g), “Luminous bacteria” (<100 cfu/g), Enterobacteriaceae (420 cfu/g) and Lactic acid bacteria (3,700,000 cfu/g). 9 isolates were identified as *Brochothrix* spp (3 isolates), Lactic acid bacteria (2 isolates), *Pseudomonas* sp. (2 isolates) and unidentified (2 isolates). One of the unidentified isolates produced ca. 8,500 mg/kg of histamine in broth at 10°C and this bacteria most likely have been responsible for histamine formation in the smoked escolar product.

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