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Changes in histamine and microbiological analyses in fresh and frozen tuna muscle during temperature abuse

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Food Types:	Fish

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1 Figure 1. Regression analysis of histamine and Aerobic Colony Counts, Enterobacteriaceae and coliforms

Table 1

Temperature abuse conditions

Trial group	Subgroup	Number of loins used	Storage temperature	Storage time (days)	Abuse temperature	Abuse duration (h day ⁻¹)	Original State	Defrosting procedure
1	Controls (C)	3	3 – 4°C	7	20°C	0	Frozen	Fridge T (3-4°C) for 24h
	1	3				2		Fridge T (3-4°C) for 24h
	2	3						Room T (18-22°C) for 12h
	3	3						MW (20 min at defreezing). Surface T = 15-18°C, Core T=3-5°C
2	Controls (C)	3	0 – 2°C	12	30°C	0	Fresh	
	1	3				2		
	2	3					Frozen	Fridge T (3-4°C) for 24h
3	Controls (C)	3	6 – 7°C	12	30°C	0	Fresh	
	1	3				2		
	2	3					Frozen	Fridge T (3-4°C) for 24h

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Table 2

Histamine concentrations and bacterial counts in tuna loins stored at 3-4°C and abused at 20°C (histamine (ppm) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets).

		Days	H ^a	ACC ^b	E ^c	C ^d	ST ^e	SRB ^f	LAB ^g	PS ^h
Storage at 3 – 4°C 2 h of temperature abuse at 20°C	Controls	0	11.4 (3.3)	3.1 (0.1)	2.9 (0.2)	2.3 (0.1)	<2	<1	<2	2
		5	31 (2.5)	5.4 (1.7)	3.5 (1.1)	1.7 (1.2)	3.4 (2.4)	1.15 (0.8)	3.4 (2.4)	5.5 (0.9)
		7	30 (6.1)	6.3 (1.1)	4 (1.2)	2.7 (1.9)	4.9 (1.6)	<1	3.6 (2.6)	5.9 (1.5)
	Defrosting at fridge temperature									
		0	17.1 (0.5)	3.8 (0.4)	3.4 (0.2)	3.1 (0.3)	2.2 (0.9)	0.6 (0.5)	1.3 (1)	2.2 (1.1)
		5	30.3 (0.6)	6.2 (0.5)	3.2 (0.4)	3.2 (0.5)	6 (0.3)	0.8 (0.6)	5.2 (0.6)	6.5 (0.2)
		7	29.1 (1.7)	6.9 (0.5)	5.1 (0.6)	4.4 (0.7)	6.8 (0.2)	0.3 (0.3)	5.8 (0.6)	7.1 (0.5)
	Defrosting at room temperature									
		0	20.4 (0.6)	4.1 (0.8)	2.8 (0.4)	2.5 (0.2)	3.3 (0.6)	<1	2.2 (1.1)	3.3 (1.6)
		5	32 (8.5)	6.4 (0.4)	5.1 (0.6)	3.3 (1.3)	5.9 (0.7)	1.4 (0.7)	5.4 (0.4)	6.4 (0.4)
		7	36 (3.5)	8.1 (0.1)	6.6 (0.4)	3.9 (0.8)	6.6 (0.6)	<1	6.5 (0.5)	8 (0.3)
	Defrosting using a microwave oven									
		0	16.6 (3.7)	4 (0.8)	3.5 (0.4)	2.8 (0.2)	1.1 (0.9)	<1	1.6 (0.6)	3.1 (1.5)
		5	28.2 (6.5)	5.4 (1)	4.6 (0.7)	1.7 (1.4)	4.7 (1.1)	1.6 (0.7)	3.4 (1.4)	6.6 (0.3)
		7	63 (26.4)	7 (0.8)	5.1 (1.4)	3.6 (1.4)	4.9 (2)	0.5 (0.4)	4.3 (1.9)	6.8 (0.7)

^aHistamine, ^bAerobic colony count (30°C), ^cEnterobacteriaceae, ^dColiforms, ^e*Staphylococcus* spp., ^fSulfite reducing bacteria, ^gLactic acid bacteria, ^h*Pseudomonas* spp.

Deleted: ., ⁱ*Vibrio* spp.,
^j*Photobacterium* spp

Table 3

Histamine concentrations and microbial counts in fresh and frozen tuna loins stored at 0-2°C and abused at 30°C (histamine (ppm) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets).

	Days	H ^a	ACC ^b	E ^c	C ^d	ST ^e	SRB ^f	LAB ^g	PS ^h
Storage at 0-2°C 2 h of temperature abuse at 30°C	Controls								
	0	20.1 (18.6)	4.8 (0.3)	4 (0.3)	2.9 (0.3)	3.5 (0.1)	1.5 (0.3)	3.5 (0.1)	3.8 (0.3)
	4	20.5 (2.5)							
	8	50.7 (5.9)	6.8 (0.3)	5 (0.3)	4.2 (0.2)	5 (0.7)	1.2 (0.2)	5.4 (0.1)	5.9 (0.1)
	12	33.5 (1.8)	7.4 (0.2)	6 (0.1)	2.3 (1.6)	6.4 (0.6)	1.3 (0.2)	6.5 (0.2)	6.4 (0.1)
	Fresh tuna								
	0	23.3 (10.2)	5.6 (0.3)	4.1 (0.1)	3.5 (0.2)	4.4 (0.5)	1.7 (0.5)	4 (0.6)	4.6 (0.3)
	4	30.6 (6.6)							
	8	80.9 (30.6)	7.1 (0.2)	5.2 (0.5)	4.2 (0.4)	5.5 (0.3)	1.6 (0.6)	6.2 (0.1)	6.3 (0.4)
	12	258.3 (79.2)	7.1 (0.2)	6 (0.6)	3.8 (1.6)	6.4 (0.4)	2.3 (0.2)	6.7 (0.3)	6.7 (0.3)
	Frozen tuna								
	0	28.8 (3.5)	4.8 (0.3)	3 (0.4)	2.7 (0.4)	3.6 (0.3)	1.7 (0.2)	3.7 (0.2)	4.2 (0.5)
	4	50.5 (3)							
	8	24 (2)	7.3 (0.1)	5 (0.2)	4 (0.2)	6.6 (0.3)	3 (0.4)	6.4 (0.3)	6.8 (0.3)
	12	32.3 (6.4)	7.7 (0.2)	5.5 (0.1)	4.3 (0.3)	7.3 (0.5)	3.1 (0.2)	7 (0.4)	6.7 (0.1)

^aHistamine, ^bAerobic colony count (30°C), ^cEnterobacteriaceae, ^dColiforms, ^e*Staphylococcus* spp., ^fSulfite reducing bacteria, ^gLactic acid bacteria, ^h*Pseudomonas* spp.

Deleted: ., ⁱ*Vibrio* spp.,
^j*Photobacterium* spp

Table 4

Histamine concentrations and microbial counts in fresh and frozen tuna loins stored at 6-7°C and abused at 30°C (histamine (ppm) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets).

		Days	H ^a	ACC ^b	E ^c	C ^d	ST ^e	SRB ^f	LAB ^g	PS ^h
Storage at 6-7°C	Controls	0	14.3 (2.6)	4.8 (0.3)	3.5 (0.2)	2.9 (0.2)	1.9 (0.8)	0.3 (0.3)	4.7 (0.2)	3 (0.2)
		4	48.2 (19.7)							
		8	147.3 (46.6)	7 (0.3)	4.6 (0.2)	3.6 (0.2)	5.7 (0.4)	1.7 (0.7)	7.2 (0.3)	5.2 (0.9)
		12	350 (95.6)	7.2 (0.1)	4.8 (0.1)	3.8 (0.5)	5.9 (0.2)	1.6 (0.6)	7.6 (0.2)	7.2 (0.3)
	2 h of temperature abuse at 30°C	Fresh tuna								
		0	26.7 (7.72)	4.6	2.8 (0.4)	1.5 (0.6)	1.2 (1)	<1	5.1 (0.5)	1.4 (0.6)
		4	429.6 (316.8)							
		8	769 (398)	7.1 (0.3)	4.9 (0.5)	4.4 (0.7)	5.5 (0.2)	0.3 (0.3)	6.3 (0.2)	5.5 (0.2)
		12	1962 (908.6)	7.9 (0.2)	5.8 (0.4)	5.1 (0.8)	6.2 (0.4)	1.6 (0.7)	7.8 (0.5)	6.9 (0.1)
		Frozen tuna								
		0	35.1 (10.6)	4.6 (0.5)	3.5 (0.1)	0.9 (0.7)	3.2 (0.3)	0.3 (0.3)	3.8 (0.2)	2.2 (0.2)
		4	921.6 (720.3)							
		8	318.5 (214.4)	7.4 (0.1)	5.8 (0.3)	4.1 (0.8)	6.4 (0.2)	0.3 (0.3)	6.6 (0.2)	6.1 (0.3)
		12	1375.6 (550.3)	7.6 (0.1)	6.42 (0.3)	4.9 (0.5)	6.9 (0.2)	3 (0.3)	7.2 (0.3)	6.7 (0.3)

^aHistamine, ^bAerobic colony count (30°C), ^cEnterobacteriaceae, ^dColiforms, ^e*Staphylococcus* spp., ^fSulfite reducing bacteria, ^gLactic acid bacteria, ^h*Pseudomonas* spp.

Deleted: ⁱ*Vibrio* spp.,
^j*Photobacterium* spp.

Table 5

Bacterial isolates and histamine concentration in culture supernatants after incubation at 37°C for 48 h.

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		Number of isolates	Strain	[Histamine (ppm)]			Number of isolates	Strain	[Histamine (ppm)]
Enterobacteriaceae					Gram positive cocci				
<i>Citrobacter</i>	<i>braaki</i>	2	C1	13	<i>Staphylococcus aureus</i>		1	S3	0
			C5	4	<i>auricularis</i>		1	S6	0
	<i>freundii</i>	7	C2	42	<i>epidermidis</i>		4	S1	0
			C3	19				S2	0
			C4	8				S5	0
			C6	6				S11	0
			C7	5	<i>hominis</i>		4	S4	305
			C8	2				S7	1
			C9	2				S9	0
<i>Enterobacter</i>	<i>amnigenus</i>	2	E3	0				S10	0
			E6	0	<i>simulans</i>		1	S15	1
	<i>aerogenes</i>	1	E4	60	<i>xylosus</i>		5	S8	1
	<i>cloacae</i>	7	E1	2				S12	0
			E2	0				S13	0
			E5	0				S14	0
			E6	0				S15	0
			E7	0	<i>Enterococcus faecalis</i>		1	EN1	15
<i>Escherichia</i>	<i>coli</i>	4	ES1	20	<i>hirae</i>		1	EN2	2404
			ES2	18	Gram negative rods				
			ES3	3	<i>Burkholderia cepacia</i>		1	B1	0
			ES4	2	<i>Flavimonas oryzihabitans</i>		1	F1	0
<i>Ewingella</i>	<i>americana</i>	1	EW1	1	<i>Pseudomonas aeruginosa</i>		1	P4	1
<i>Hafnia</i>	<i>alvei</i>	2	H1	76	<i>fluorescens</i>		6	P1	1
			H2	66				P2	0
<i>Klebsiella</i>	<i>oxytoca</i>	2	K3	2156				P4	0
			K5	2149				P7	0
	<i>terrigena</i>	1	K2	0				P9	0
	<i>pneumoniae</i>	3	K1	0				P10	0
			K4	0	<i>putida</i>		5	P3	2
			K6	0				P5	1
<i>Morganella</i>	<i>morganii</i>	4	M1	20				P6	0
			M2	6875				P8	0
			M3	6296	<i>Aeromonas sobriae</i>		1	A1	7
			M4	24499	<i>caviae</i>		1	A2	0
<i>Proteus</i>	<i>mirabilis</i>	2	P1	11	<i>Pasteurella haemolytica</i>		2	PA1	0
			P3	8				PA2	0
	<i>vulgaris</i>	2	P2	0					
			P4	0					
<i>Rahnella</i>	<i>aquatilis</i>	2	R1	0					
			R2	0					
<i>Salmonella</i>	<i>poona</i>	1	SA1	14					

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Number of loins used	Storage temperature	Storage time (days)	Abuse temperature	Abuse duration (h day ⁻¹)	Original State	
3	3 – 4°C	7	20°C	0	Frozen	Fridge Trial group
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						Fridge
						Trial group
3	3 – 4°C	7	20°C	0	Frozen	
3				2		
3						
3	0 – 2°C	12	30°C	0	Fresh	
3				2	Frozen	
3	6 – 7°C	12	30°C	0	Fresh	
3				2	Frozen	

Changes in histamine and microbiological analyses in fresh and frozen tuna muscle during temperature abuse

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Running title: Histamine and bacterial flora in tuna fish

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Abstract

Temperature abuse of tuna (*Thunnus alalunga*) was carried out in order to assess the histamine build-up in fish processing facilities where fish can be exposed to high temperatures for short periods of time. Histamine production was studied in tuna loins under different storage and abuse conditions. Tuna was stored at 0-2°C, 3-4°C and 6-7°C and abused for 2 h daily at 20°C and 30°C for 7-12 days. Loins abused at 30°C for 2 h daily, contained potentially toxic histamine concentrations (67–382 mg kg⁻¹) when stored at low refrigeration temperature (0-2°C), whereas when stored at 6-7°C the loins contained highly toxic histamine concentrations (544.5–4156.6 mg kg⁻¹). Lower histamine concentrations (23 to 48 mg kg⁻¹ in loins stored at 0-2°C and 124.7–2435.8 mg kg⁻¹ in loins stored at 6-7°C) were observed in temperature abused loins that were initially frozen. An increase over time was observed in most microbial counts tested. Bacteria isolated from the temperature abused loins showed varied ability of histamine production with *Morganella morganii*, *Klebsiella oxytoca*, *Staphylococcus hominis* and *Enterococcus hirae* being the most active histamine producing bacteria.

Keywords: Histamine, tuna, storage, temperature abuse, bacteria.

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1 Introduction

2
3 Histamine formation in fish is related to the free histidine content of the fish muscle,
4 the presence of bacterial histidine decarboxylases and certain environmental
5 conditions. The production of histamine is largely induced by post-harvest exposure
6 to high temperature of the fish. Temperature enhances the multiplication of bacteria
7 and the bacterial production of histidine decarboxylases. In addition, histidine
8 decarboxylase activity is favoured by the exposure to temperatures higher than 15°C
9 (Lehane and Olley 2000; Kanki et al. 2007). In general, histamine production is
10 favored by temperatures higher than 5°C with an optimum of 20 – 30°C (Kim et al.
11 2000; Lehane and Olley 2000), and by the presence of bacterial species that are
12 prolific histamine producers. Enterobacteriaceae (*Morganella morganii*, *Enterobacter*
13 *cloacae*, *Citrobacter freundii*, *Escherichia coli*, *Hafnia alvei*, *Klebsiella pneumoniae*,
14 *Proteus* spp., *Serratia macrescens*) have been reported to be the most prolific
15 histamine producers (Taylor et al. 1979; Behling and Taylor 1982; Taylor 1986;
16 Klausen and Huss 1987; Ababouch et al. 1991; Rodriguez-Jerez et al. 1994; Emborg
17 and Dalgaard 2006), followed by the lactic acid bacteria (*Lactobacillus* spp.,
18 *Carnobacterium* spp.) (Taylor 1985; Joosten and Northolt 1989) and *Photobacterium*
19 spp. (Ababouch et al. 1991; Silla Santos 1996; Dalgaard et al. 2006; Kanki et al.
20 2007). Other bacterial genera such as *Staphylococcus* spp., *Pseudomonas* spp.,
21 *Aeromonas* spp., *Plesiomonas shigelloides* and *Clostridium perfringens* have also
22 been implicated in SFP (Hernández-Herrero et al. 1999). *Vibrio* spp. used not to be
23 considered histamine producers; however there are recent reports on *V. alginolyticus*
24 and *V. anguillarum* for being weak histamine producers (Actis et al. 1999; Kim et al.

2001). The histamine-producing bacteria are mostly common post-catching contaminants of fish (Hernández-Herrero et al. 1999).

Customarily in kitchens (either professional or household) the environmental conditions are far from the ideal concerning environmental temperature, preparation time and hygiene. Frequently there is a crucial lag phase from defrosting and taking a tuna loin out of the refrigerator to preparing (cutting in steaks, cubes etc) and cooking it. Furthermore ambient storage temperatures accelerate bacterial growth and since most prolific histamine producers are common food contaminants, high storage temperature and feebly handling procedures can result in the production of histamine at toxic levels.

Scombroid or Histamine fish poisoning (SFP, HFP) is one of the most frequently reported intoxications associated with the consumption of fish worldwide. The SFP intoxication is generally related with high levels of histamine in spoiled fish. Histamine, a biogenic amine involved in allergic reactions, is the main toxin involved in SFP and its toxicity is enhanced by the presence of other biogenic amines in foods (Lehane and Olley 2000). Histamine is formed by decarboxylation of histidine, which is found in high levels in the muscle of fish belonging to the Scomberidae and Scomberesocidae families such as tuna, albacore, mackerel and bonito. Still non-scombroid fish such as mahi-mahi, sardines, pilchards, anchovies, herring and marlin have frequently been implicated in SFP (Taylor 1985; Clifford and Walker 1992).

Symptoms of SFP are cardiovascular, gastrointestinal and neurological, including skin rashes, urticaria, oedema, localized inflammation, nausea, vomiting, diarrhea, cramping, hypotension, headaches, palpitations and an oral burning and

blistering sensation (Lehane and Olley 2000; Gillespie et al. 2001). The SFP intoxication has to be differentiated from other food allergies. Manifestation and severity of symptoms may vary according to individual susceptibility, dietary components and medications, such as isoniazid and antimalarials, which may increase susceptibility. The incubation period is short (less than 2 h from consumption of food) and symptoms usually resolve within a few hours (Lerke et al. 1978; Taylor 1986; Lehane and Olley 2000; McLauchlin et al. 2005).

Histamine is not inactivated by the usual thermal processes of the contaminated food (freezing, chilling, cooking, canning etc) and the sole prevention is food storage at temperatures below 4°C and avoidance of prolonged handling at ambient temperatures. The amount of histamine produced is a function of the fish species, the individual fish and the fish part which is sampled, the time, the temperature and the type and number of bacteria species present. The toxic dose is variable. Recent reviews of the oral toxicity of histamine to humans in different foods concluded that SFP is slight at 8-40 mg total dose, moderate at >40 mg and severe at >100 mg (Shalaby 1996; Lehane and Olley 2000). Scoging (1998a) analysed 405 incidents of SFP in the UK and suggested the following classification: <100 mg kg⁻¹, safe for consumption; 100-500 mg kg⁻¹, mishandled and potentially toxic; 500-1000 mg kg⁻¹, unsatisfactory and probably toxic; >1000 mg kg⁻¹, toxic and unsafe for consumption. According to the European legislation (Council Directive, 1991) among nine samples from each batch the mean concentration of histamine must not exceed 100 mg kg⁻¹; two samples may have a concentration of histamine of more than 100 mg kg⁻¹ but less than 200 mg kg⁻¹ and no sample may have a concentration of histamine exceeding 200 mg kg⁻¹.

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2 Tuna fish is considered to be susceptible to histamine formation. Fresh tuna has a
3 shelf life of about 11 days from when they are caught when stored at temperature
4 $>4^{\circ}\text{C}$ (Anonymous 1997). In recent years there are an augmenting number of
5 incidents of SFP attributed to consumption of fish (mainly tuna) in restaurants
6 (Anonymous 1986; Scoging 1998a; 1998b; Predy 2003; Wu and Chen 2003; Hall
7 2003; Feldman et al. 2005; McLauchlin et al. 2005; Sigmundsdóttir et al. 2005),
8 where high concentrations of histamine are recorded in fish stored at appropriate
9 temperatures but thawed at the usually high room temperatures of the kitchen. Little
10 information is available about the effects of short – term temperature abuse and
11 bacterial flora on histamine production in tuna fish at the end-point (kitchen). The
12 objective of our study was to assess the effect of different temperature regimens,
13 simulating refrigerated storage and short – term temperature abuse, on histamine
14 formation together with the development of histamine forming microflora in tuna
15 steaks and loins, regardless of the temperature treatment they are subjected before
16 they reach a restaurant kitchen. This simulation of malpractices during the
17 preparation of tuna meals at a restaurant kitchen could be helpful to assess the risks
18 for public health and to implement proper control measures. Also an evaluation of the
19 histamine production capability of the different bacteria comprising the microflora of
20 spoiled fish was performed.

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3 1 **Materials and methods**
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8 3 **Samples**
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12 5 The fish samples used for the trials were 30 tuna loins (*Thunnus alalunga*) collected
13 6 by Sampling Officers from consignments landed at Heathrow Airport. Six of the tuna
14 7 loins were imported from Brazil, 6 from Sri-Lanka, 6 from Maldives, 8 from Indonesia
15 8 and 4 from Yemen. The specimens were delivered to the Food Safety Microbiology
16 9 Laboratory (FSML), Central Public Health Laboratory Service (CPHLS) within 6 h
17 10 after collection. The average weight of the loins was 2.64 kg, ranging from 2.14 kg to
18 11 3.21 kg. The fresh loins were transported to the laboratory in insulated boxes and
19 12 reached the laboratory 1 to 2 days post catching. Every effort was made to keep the
20 13 temperature of storage of fresh loins at 3-4°C from the point of collection to the use of
21 14 the specimens for the temperature abuse trial. 18 of the loins used (loins from Brazil,
22 15 Sri – Lanka and Maldives) were frozen at the place of origin. They were transported
23 16 at the laboratory frozen in well insulated boxes and stored until defrosting in a
24 17 temperature monitored freezer at -35°C. All tuna fish samples were skinned and
25 18 placed into sterile plastic bags prior to any processing. For all samples the histamine
26 19 and microbiological analysis was performed the day of arrival at the laboratory to
27 20 ensure good quality of the loins. The temperature abuse trial has taken place one
28 21 day after the arrival of specimens to the laboratory.
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55 23 **Temperature abuse process**
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57 24 The tuna loins were divided into 3 groups. One group was stored at 3-4°C and
58 25 consisted of 12 frozen (-35°C) loins, divided into 4 subgroups (3 trial subgroups
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1 containing 3 loins each and one control subgroup containing 3 loins). The control
2 subgroup was defrosted at 3-4°C and received no further treatment. The rest 3
3 subgroups were defrosted using 3 different regimes: at 3-4°C in a temperature
4 monitored refrigerator, at 20°C (room temperature) on the lab bench and in a
5 microwave oven (Philips Cooktronic M710, Philips Electronics UK Ltd, UK) according
6 to the manufacturer's instructions. The 3 trial subgroups were temperature abused
7 for 2 h daily at 20°C for 7 consequent days. Histamine concentration and
8 microbiological tests were performed prior to temperature abuse and on the 5th and
9 7th day of temperature abuse.

11 The second group of loins was stored at 0-2°C in ice inside a temperature monitored
12 refrigerator and consisted of 9 loins (6 fresh reserved at 3-4°C, 3 frozen reserved at -
13 35°C). The 3 out of the 6 fresh loins served as controls and received no further
14 treatment, while the rest 3 fresh loins were temperature abused for 2 h daily at 30°C
15 in a temperature monitored incubator for 12 consequent days. The 3 frozen loins
16 were defrosted at refrigeration temperature (3-4°C) in a temperature monitored
17 refrigerator. All loins were tested for histamine prior to temperature abuse and on the
18 4th, 8th and 12th day of temperature abuse and were microbiologically analyzed prior
19 to temperature abuse and on the 8th and 12th day of temperature abuse.

21 The third group was stored at 6-7°C using a cooled incubator (LMS Cooled incubator,
22 model No 303, LMS Ltd, UK) and consisted of 9 loins (6 fresh reserved at 3-4°C, 3
23 frozen reserved at -35°C) divided into 3 subgroups. One subgroup of 3 fresh loins
24 served as control and received no further treatment, while the other subgroup of 3
25 fresh loins was temperature abused for 2 h daily at 30°C in a temperature monitored

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3 1 incubator for 12 consequent days. The subgroup of 3 frozen loins was defrosted at
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5 2 refrigeration temperature in a temperature monitored refrigerator. Histamine
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7 3 concentration was tested prior to abuse and on the 4th, 8th and 12th day of
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9 4 temperature abuse, while microbiological analysis was performed prior to abuse and
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11 5 on the 8th and 12th day of temperature abuse. The temperature abuse conditions are
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13 6 concisely presented in Table 1.
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20 8 ***Sampling for microbiological and histamine analysis***

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22 9 Sampling for microbiological and histamine analysis was carried out from the surface
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24 10 of the middle part of the loin, from ~1cm depth, by cutting aseptically a vertical strip
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26 11 weighing at least 30 g. The sample was homogenized in a laboratory blender (MSE
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28 12 Atomix). 10 g were used for the microbiological analyses and 10 g for the histamine
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30 13 analysis. Samples during the temperature abuse trial were taken from almost
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32 14 adjacent areas so as to ensure uniformity in initial histamine content and microbial
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34 15 populations.
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41 17 ***Microbiological analysis***

42 18 ***Bacterial enumeration***

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44 19 Bacterial counts were restricted to routinely detected food contaminants, which are
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46 20 also supposed to be histamine producers (Enterobacteriaceae, *Pseudomonas* spp.,
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48 21 *Staphylococcus* spp., sulfite reducing bacteria, lactic acid bacteria, *Photobacterium*
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50 22 spp. and *Vibrio* spp.) and was carried out in standard selective media. Ten grams of
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52 23 homogenized muscle was combined with 90 ml of maximum recovery diluent and
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54 24 ten-fold serial dilutions were performed. For surface plating 0.1 ml of each dilution
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56 25 was used and for pour plating 1 ml of each dilution was used.
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6 2 Aerobic colony counts (ACC) were determined on Plate Count Agar using surface
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8 3 plating after incubation for 2 days at 30°C. Enterobacteriaceae, were determined on
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10 4 MacConkey agar No.3 using surface plating after incubation for 1 day at 37°C.
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12 5 MacConkey agar No.3 permits the growth of other bacteria, so the estimated counts
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14 6 should be considered as presumptive Enterobacteriaceae. *Staphylococcus* spp. were
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16 7 determined on Baird-Parker agar with egg yolk-tellurite supplement by surface plating
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18 8 after incubation at 37°C for 2 days. Characteristic colonies were tested for catalase
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20 9 and coagulase production (Staphytect plus, Oxoid Ltd, Hampshire, England).
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22 10 *Pseudomonas* spp. were determined using *Pseudomonas* selective agar
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24 11 supplemented with Cetrimide (10 mg l⁻¹) – Fucidin (10 mg l⁻¹) – Cephaloridine (50
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26 12 mg l⁻¹) by surface plating after incubation for 2 days at 30°C. Characteristic colonies
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28 13 were confirmed as *Pseudomonas* spp. by testing for oxidase production with the use
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30 14 of oxidase identification sticks (Oxoid Ltd, Hampshire, England). Sulfite reducing
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32 15 bacteria (SRB) were determined on Tryptose Sulfite Cycloserine agar supplemented
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34 16 with Perfringens (TSC) selective supplement (D-cycloserine 400 mg l⁻¹; Oxoid Ltd,
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36 17 Hampshire, England) using a pour plate with an overlay after incubation for 2 days at
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38 18 37°C in an anaerobic cabinet (Modular Atmosphere Controlled System, Don Whitley
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40 19 Scientific Ltd, West Yorkshire, UK). Lactic acid bacteria (LAB) were determined on
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42 20 DeMan-Rogosa-Sharpe agar by surface plating after incubation for 3 days at 37°C in
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44 21 an anaerobic cabinet. For the determination of *Vibrio* spp. and *Photobacterium* spp.
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46 22 ten fold serial dilutions were prepared in Alkaline peptone water (APW). 0,1 ml of
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48 23 each dilution was plated on Thiosulphate Citrate Bile – salt Sucrose (TCBS) agar and
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50 24 incubated at 37°C for 24 h for the detection of *Vibrio* spp. and 20°C for 48 h for the
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52 25 detection of *Photobacterium* spp. (Holt et al, 1994). In addition, Gram staining

prepared from randomly selected colonies was performed. The methodology followed is described by Roberts et al. (1995). All media and broths were supplied by the PHLS Media Department (CPHLS Media Department, Colindale, London, UK) (ISO 9002 standard) and were quality controlled by the Media Department Quality Control to UKAS standards.

Isolation of bacteria

Characteristic colonies were picked from the selective media used and were characterized to the genus level. All isolates were pure cultured and characterized Gram stain was performed so as to test culture purity and the morphology of the isolates.

The identification of the bacteria isolated from MacConkey agar was carried out with the use of the API 20 E system (20 100, BioMérieux, Marcy-l'Etoile, France). The tests used for the identification of *Staphylococcus* spp. were the catalase test, the coagulase test (Staphytest plus, DR850M, Oxoid Ltd, Hampshire, England), the DNase production test (DNAase agar, Scharlau Chemie S.A., Sentmenat, Barcelona, Spain), the ability of the isolates to grow in Nutrient agar (Scharlau Chemie S.A., Sentmenat, Barcelona, Spain) supplemented with 7,5% NaCl and 10% NaCl and the API 20 STAPH system (20 500, BioMérieux, Marcy-l'Etoile, France). *Pseudomonas* spp. were identified according to their ability to produce oxidase (oxidase identification sticks, BR64A, Oxoid Ltd, Hampshire, England) and with the use of the API 20 NE system (20 050, BioMérieux, Marcy-l'Etoile, France). The identification of *Vibrio* spp. and *Photobacterium* spp. was carried out with the use of the oxidase test, the catalase test (Hydrogen peroxide 30%, analysis grade, 107209 Merck KgaA,

Darmstadt, Germany), the inhibitory effect of the vibriostatic agent O129 (10 µg, DD14 and 150 µg, DD15, Oxoid Ltd, Hampshire, England and the use of the API 20NE system (20 050, BioMérieux, Marcy-l'Etoile, France).

The bacterial isolates were stored until the examination of their histamine production ability in beads of the Microbank system (ProLab Diagnostics, Ontario, Canada)

Histamine production by bacterial isolates

The ability of the isolated bacteria to produce histamine was measured in the culture supernatant of Møller decarboxylase broth (MD broth) (Atlas, 1995). Prior to the inoculation in MD broth the isolates were inoculated to Trypticase Soy broth containing histidine (TSBH).

TSBH was prepared by addition of 30 g of Tryptone soy broth (Tryptone Soya Broth (U.S.P.) CM0876, Oxoid Ltd, Wade Road, Basingstoke, UK), 1 g of histidine (L-Histidine dihydrochloride, Sigma – Aldrich) in 1 l of deionized water. The pH of TSBH was adjusted to pH 6.8 before autoclaving for 10 min at 121 °C.

The composition of MD broth was tryptone 0,5% (EZMix Tryptone, T-2559, Sigma Chemical Co, St. Louis, USA), beef extract 0,5% (B-4888, Sigma Chemical Co, St. Louis, USA), glucose 0,05% (α-D-Glucose analytical grade, 17023051, SERVA Electrophoresis GmbH, Heidelberg, Germany), bromocresol purple 0,001% (1.03025, Merck KgaA, Darmstadt, Germany), cresol red 0,0005% (11,447,2, Aldrich Chemical Co, Milwaukee, USA), pyridoxal phosphate 0,0005% (pyridoxal 5'-phosphate, P-9255, Sigma) and histidine 1%. The pH of MD broth was adjusted to pH 6.0 before autoclaving for 15 min at 121 °C.

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2 The isolates were inoculated in TSBH and incubated at 37°C for 24 h. 100 µl of the
3 culture was inoculated in MD broth containing 1% histidine and incubated at 37°C for
4 48 h. After 48 h the culture supernatant was collected and centrifuged 2 times at
5 3500 mg kg⁻¹ in a desktop centrifuge (Sorvall GLC-4, Sorvall Instruments,
6 Wilmington, DE, USA). The supernatant was sterilized with the use of Acrodisc
7 syringe filters 0.45 µm (Pall, New York, USA). The filtered culture supernatants were
8 stored at – 85°C until histamine analysis.

10 ***Histamine analysis***

11 For the histamine analysis of tuna a fluorometric method was performed as described
12 by Taylor (1978). 10 g of fish flesh were mixed with 50 ml methanol (822283, Merck
13 KgaA, Darmstadt, Germany) and were incubated at 60°C in a water bath (type W760,
14 Memmert GmbH, Schwabach, Germany) for 15 min. 6 ml of water saturated butanol
15 (101990, Merck KgaA, Darmstadt, Germany), 2 g of sodium carbonate decahydrate
16 (106391, Merck KgaA, Darmstadt, Germany) and 1 ml of 5M NaOH (Sodium
17 hydroxide granulated, 106469, Merck KgaA, Darmstadt, Germany) was added to the
18 properly diluted methanol extract of the fish flesh. The tubes were shaken and were
19 allowed to settle. 3 ml of the organic phase was mixed with 3 ml of 0.1 M HCl
20 (320331, ACS reagent 37%, Aldrich Chemical Co, Milwaukee, USA). The organic
21 phase was discarded. 0.4 ml of 1M NaOH and 0.1 ml of 1% o-phthalaldehyde (P
22 0657, Sigma Chemical Co, St. Louis, USA) were added and fluorescent output was
23 measured by a fluorimeter (Kontron M25, Kontron Analytical, Kontron Instruments
24 Ltd, Herts, UK) at 360 nm excitation and 450 nm emission wavelengths. Histamine

1 concentration levels were measured prior to any procedure and after time-
2 temperature abuse of the samples.
3

4 For the histamine analysis of culture supernatants a commercial ELISA kit for the in-
5 vitro diagnostic quantitative determination of histamine in human plasma and urine
6 and in cell culture supernatants was used (Histamine ELISA, RE 592 21, IBL-
7 Hamburg GmbH, Hamburg, Germany). Histamine analysis was performed according
8 to the manufacturer's instructions.
9

10 Prior to histamine analysis of culture supernatants, a validation of the kit was
11 performed. 5 sterile spiked samples of the broth containing different histamine
12 concentrations (0, 5, 20, 50 and 100 mg kg⁻¹) were analysed five times. The results
13 were sufficient for the use of this kit in the measurement of histamine in culture
14 supernatants. A plot of the measured histamine concentration against the actual
15 histamine concentration was drawn. The regression factor of the measured histamine
16 was $r^2 = 0,982$ and the difference between the measured and the actual
17 concentration of histamine did not exceed 2 standard deviations. These results were
18 judged as adequate for the employment of this method in the measurement of
19 histamine in MD broth supernatants.
20

21 ***Statistical analysis***

22 The two-sided student t-test was used. Descriptive statistics of means, standard
23 deviation and standard error were applied in analyzing the results. A significance
24 level of 5% was used. The regression analysis between histamine concentration and
25 bacterial counts was contacted by plotting scattered graphs and calculating the r^2

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factors. r^2 factors higher than 0.5 were accepted to indicate a correlation between the histamine concentration and the bacterial counts measured.

Results

Temperature abuse

The histamine concentrations of the tuna samples upon delivery were ranging from 1 mg kg⁻¹ to 47.1 mg kg⁻¹. The histamine concentrations of the tuna during the time-temperature abuse procedure are presented in Tables 2 – 4.

In defrosted loins stored at 3 – 4°C, histamine concentrations were low (<60mg kg⁻¹) both in controls and loins abused daily at 20°C for 2 h (Table 2), with only one abused frozen loin exceeding this level (127.4 mg kg⁻¹). There were only minor differences between the different defrosting regimes, and all loins were characterized according to their histamine content as safe for consumption at the end of the abuse procedure.

In loins stored at 0–2°C the histamine concentration was low (<70mg kg⁻¹) both in controls and defrosted loins abused daily at 30°C for 2 h. Higher concentrations (67–382 mg kg⁻¹) were measured in fresh loins abused daily at 30°C for 2h (Table 3). However, the increase was not statistically significant ($p>0.10$) and at the end of the abuse procedure the controls and defrosted loins were characterized safe for consumption whereas fresh loins were potentially toxic.

In loins stored at 6-7°C there was a significant increase ($p<0.05$) in histamine levels in the controls, resulting in final concentrations of 228.3–584.2 mg kg⁻¹ (Table 4).

1 The final concentration of histamine in defrosted loins abused daily at 30°C for 2 h
2 (124.7–2435.8 mg kg⁻¹) was higher than the histamine concentration of the controls.
3 In fresh temperature-abused loins, histamine increased to final concentrations 544.5–
4 4156.6 mg kg⁻¹. At the end of the abuse procedure, both the controls and the
5 temperature abused loins were characterized as unsuitable for consumption, while
6 temperature – abused fresh loins being highly toxic.

7
8 No *Vibrio* spp. and *Photobacterium* spp. counts were recorded and no strains of
9 these species were isolated, although cultures of these species were performed
10 throughout the experimentations. In temperature abused loins and controls stored at
11 3 – 4°C (Table 2) the counts of *Enterobacteriaceae* and coliforms increased by 2.5–
12 3.2 log₁₀ and 2.1–2.8 log₁₀ respectively whereas the ACC, LAB and *Pseudomonas*
13 spp. counts increased by approximately 3 log₁₀ (2.7 – 4.5 log₁₀, 3.1–5 log₁₀ and 1.5–
14 5.7 log₁₀ respectively) after seven days of storage. However, minimal changes were
15 observed in the counts of SRB in this group of tuna loins.

16
17 In temperature abused loins and controls stored at 0–2°C (Table 3), the counts of
18 *Enterobacteriaceae* and coliforms increased by approximately 2 log₁₀ (1.9–2.7 log₁₀
19 and 1.1–2.4 log₁₀ respectively). The ACC, *Staphylococcus* spp., LAB and
20 *Pseudomonas* spp. counts increased by 1.4–2.6 log₁₀, 2–4.4 log₁₀ and 2.2–2.3 log₁₀
21 respectively. In this group of abused tuna loins an increase of almost 1 log₁₀ was
22 observed in the counts of SRB. There was no difference in counts between controls
23 and temperature-abused loins.

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3 1 In temperature abused loins and controls stored at 6–7°C (Table 4), the
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5 2 *Enterobacteriaceae* and coliforms counts increased by the end of the procedure by
6
7 3 approximately 3 log₁₀ (3.2 log₁₀ and 3.5 log₁₀ respectively) in the loins and by 1 log₁₀
8
9 4 in the controls. Also an increase was observed in ACC and *Staphylococcus* spp.
10
11 5 counts (3–4.1 log₁₀) LAB (2.6–3.4 log₁₀) and *Pseudomonas* spp. (2.5–5.5 log₁₀).
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18 7 Regression analysis of histamine concentrations and bacterial counts of loins stored
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20 8 at 6 – 7°C revealed correlation between histamine concentrations and aerobic colony
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22 9 counts ($r^2=0.84$), *Enterobacteriaceae* counts ($r^2=0.86$) and coliform counts ($r^2=0.87$)
23
24 10 (Figure 1). A correlation between histamine concentrations and aerobic colony
25
26 11 counts ($r^2=0.81$), *Enterobacteriaceae* counts ($r^2=0.67$) and coliform counts ($r^2=0.62$)
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28 12 was also observed in loins stored at 3 – 4°C (Figure 1), whereas in loins stored at 0 –
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30 13 2°C no correlation was observed between histamine concentrations and bacterial
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32 14 counts (Figure 1).
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39 16 ***Bacterial isolates and their potential for histamine production***

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41 17 77 isolates were isolated from temperature abused loins with high histamine
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43 18 concentration. The isolates are shown in Table 5. Forty of them belong to
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45 19 *Enterobacteriaceae* (53% of the total isolated bacteria). These bacteria belong to the
46
47 20 species *Citrobacter* spp. (22% of *Enterobacteriaceae*), *Enterobacter* spp. (20%),
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49 21 *Klebsiella* spp. (15%), *Morganella* spp. (10%) and *Escherichia* spp. (10%). The
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51 22 *Staphylococci* that were isolated represent 21% of the total bacteria isolated and
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53 23 consisted of *Staphylococcus xylosus* (24% of *Staphylococci*), *S. epidermidis* (19%),
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55 24 *S. hominis* (19%), *S. aureus* (5%), *S. auricularis* (5%) και *S. simulans* (5%). The
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57 25 Gram negative rods represented 21% of the total bacteria isolated and consisted of
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1 *Pseudomonas fluorescens* (29% of Gram negative rods), *Pseudomonas putida*
2 (24%) *Burkholderia cepacia* (5%), *Flavimonas oryzihabitans* (5%) and *Pseudomonas*
3 *aeruginosa* (5%).

4
5 *Morganella morganii* was the most active histamine producing species among the
6 bacteria isolated. In the cultures of the three out of four *M. morganii* the
7 concentrations of histamine were 24 499, 6875 and 6296 mg kg⁻¹, with the
8 concentration of 24 499 mg kg⁻¹ being the highest measured among the tested
9 bacterial cultures. High concentrations of histamine were also measured in the
10 cultures of one *Staphylococcus hominis* isolate (3050 mg kg⁻¹), one *Enterococcus*
11 *hirae* isolate (2404 mg kg⁻¹) and two *Klebsiella oxytoca* isolates (2156 and 2149 mg
12 kg⁻¹ respectively). It should be noted that the four out of five *S. hominis* isolates
13 tested did not exhibit histamine production ability. The histamine concentration of the
14 culture supernatants of the bacterial isolates tested is shown in Table 5.

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Discussion

According to our results the temperature of abuse is the most important factor in histamine formation (Tables 2, 3, 4 and Figure 1). The loins abused at 20°C (storage at 3–4°C) showed no increase in histamine, while the loins abused at 30°C (storage at 0–2°C) presented toxic histamine levels. Histamine concentrations similar to those observed in the present study have been reported by Guizani et al. (2005) in yellowfin tuna. The histamine concentration in yellowfin tuna stored at 8°C was 150 mg kg⁻¹ after 8 days of storage and in our study it was 147.3 mg kg⁻¹ after 8 days of storage. Our results coincide with those of Kim and Price (1999), who detected low concentrations of histamine (80 mg kg⁻¹) in albacore loins stored at 0°C for 18 days. Lòpez-Sabater et al. (1996) detected histamine in large tuna fish pieces from the 4th day of storage at 8°C, measuring concentrations >2500 mg kg⁻¹ after 6 days of storage at 8°C, but no histamine was detected in large tuna fish pieces after 12 days at 0°C. Frank and Yoshinaga (1987) reported histamine concentrations 3.8 mg kg⁻¹, 5.6 mg kg⁻¹ and 169 mg kg⁻¹ in skipjack tuna loins stored at –1°C, 4.4°C and 10°C respectively after 12 to 14 days of storage, which are lower than our measurements obtained under similar conditions; however, the initial histamine concentration measured in the loins by Frank and Yoshinaga was only 1.1 mg kg⁻¹, while in our loins ranged from 1 – 47.1 mg kg⁻¹. In our study histamine concentrations in control loins stored at 0 – 2°C were similar to those reported in mackerel stored in ice (Bennour et al. 1991). In Spanish mackerel stored at 0°C, histamine concentrations were lower (mean<6 mg kg⁻¹) (Middlebrook et al. 1988).

1 In our study, histamine concentration was higher in fresh than in defrosted tuna loins,
2 although the difference was not statistically significant ($p>0.05$). The freeze –
3 defrosting process appears to reduce the bacterial cell numbers or causes sub-lethal
4 injuries to bacterial cells causing poor histamine production. Baranowski et al. (1990)
5 observed that the duration of freezing of fish is an important factor for histamine
6 formation during storage of mahi-mahi fish at 32°C for 1 day. Ben-Gigirey et al.
7 (1998) reported that the survival of bacteria involved in biogenic amine formation is
8 poor at freezing temperatures. Recently Dalgaard et al. (2006) evaluated the
9 biogenic amine formation and microbial spoilage in fresh and thawed chilled garfish,
10 concluding that histamine formation depends strongly on previous freezing of the
11 fish, which probably kills freeze – sensitive bacteria like *Photobacterium* spp. or
12 *Vibrio* spp. In our study no *Photobacterium* spp. or *Vibrio* spp bacteria were isolated.
13 Taylor and Speckhard (1983) reported that histamine-producing bacteria could not be
14 isolated from frozen tuna without using a 24 h recovery period. When Staruszkiewicz
15 et al. (2004) studied the changes in selected biogenic amines in mahi-mahi and tuna
16 held in seawater at 25 to 35°C for up to 18 h, they observed that histidine
17 decarboxylase activity was retained in some frozen fish samples and could result in
18 further increases in histamine on thawing.

19
20 According to our results (Tables 2, 3, 4 and Figure 1) the daily abuse of tuna
21 triggered the growth of bacteria. The correlation observed between histamine
22 concentration, ACC, Enterobacteriaceae and coliform counts indicate that bacteria
23 belonging to these groups are responsible for histamine production in tuna loins
24 stored at temperatures $>3^{\circ}\text{C}$. Our results coincide with the statement by Stratton and
25 Taylor (1991) that enteric bacteria, specifically *Morganella morganii*, *Klebsiella*

pneumoniae and *Hafnia alvei* are the most prolific histamine producers in fish when they are maintained at temperatures $\geq 4^{\circ}\text{C}$. The *Morganella morganii* isolates (Table 5) varied in their capacity of producing histamine; isolate M4 was the most prolific histamine producer (24 499 mg kg⁻¹), while isolate M1 was a limited histamine producer (20 mg kg⁻¹). Other researchers reported *M. morganii* as the most active histamine producer (Frank et al. 1985; Ababouch et al. 1991; López – Sabater et al. 1994; Dalgaard et al. 2006). Histamine – producing *K. oxytoca* strains have been reported by López – Sabater et al. (1994), Rodríguez – Herez et al. (1994) and Hernández – Herrero et al. (1999). Our *K. pneumoniae* strains produced no detectable amounts of histamine. According to Taylor et al. (1979) *Klebsiella pneumoniae* is capable of able histamine production, but Kanki et al. (2002) assume that Taylor (1979) has mistakenly attributed histamine production to *Klebsiella pneumoniae*, misidentifying strains of *Raoultella planticola*.

No correlation was found in loins stored at 0 – 2°C, with none of the tested bacterial counts revealing any correlation with histamine production and probably, in this group of loins, another bacterial group was responsible for the production of histamine. Okuzumi et al. (1981) have reported that Psychrophiles and especially halophilic N-group bacteria growing at 5°C can produce histamine. By the end of temperature abuse trials, the dominant microbial populations were LAB followed by the Enterobacteriaceae. Similar results have been reported by Yoshinaga and Frank (1982) and López-Sabater et al. (1996) when large fresh tuna fillets were examined. Kim and Price (1999) reported lower levels ($<10^4$ CFU gr⁻¹) of ACC in albacore stored in ice, but the temperature of storage was 15°C. Koutsoumanis et al. (1999) also reported predominance of LAB and Enterobacteriaceae in steaks of *Sparus aurata*

1 stored at 0°C and 8°C. In our study the SRB counts remained low during freezing and
2 storage at low temperatures. This observation may be attributed to the inability of
3 some *Clostridia* species (e.g. *C. perfringens*) to grow at temperatures below 15°C
4 (McClane 1997). Ben-Gigigrey et al. (1998) reported absence of SRB in fresh
5 albacore too. From the Gram positive isolates, only one strain of *Staphylococcus*
6 *hominis* (S4) and one strain of *Enterococcus hirae* (EN2) were active histamine
7 producers. Hernández – Herrero et al. (1999) reported production of histamine by *S.*
8 *epidermidis* and *S. capitis* isolated from salted fish. Tham (1988) reported production
9 of small amounts of histamine from *E. faecalis* and *E. faecium* isolated from cheese.
10 The measured histamine concentration from the EN2 isolate (2404 mg kg⁻¹) is far
11 higher from the histamine concentration reported by Tham (4 mg kg⁻¹). The
12 presence of *Enterococcus* spp. in fish is relatively rare and in most cases is attributed
13 to post – catch contamination (Gram and Huss 1996). We observed that the prolific
14 histamine producers presented only 10% of the isolates, while 39% were slow
15 producers and 51% were not histamine producing bacteria. The total histamine
16 produced by the prolific producers (47.4 mg) was almost 100 times more than the
17 histamine produced by the slow histamine producers (0.43 mg), indicating that the
18 contribution of slow histamine producing species to histamine build – up in fish flesh
19 is limited.

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21 According to our results the histamine concentration was at toxic levels in tuna
22 samples which were exposed at temperatures ≥30°C for short periods every day and
23 for longer than 3 days. Considering the public health protection, appropriate storage
24 at <4°C and proper handling of tuna fish is essential for delaying histamine formation
25 and preventing SFP outbreaks. For food safety reasons the environmental

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1 temperature of fish processing facilities should be kept cool (<20°C) and exposure to
2 ambient temperatures even for short periods should be avoided. In facilities where
3 temperature control is impossible or inadequate, tuna should be stocked up for
4 shorter periods (less than 12 days). Also, the practice of freezing the tuna prior to
5 storage at fridge temperature can restrain histamine production.

6
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Captions

- Table 1. Temperature abuse conditions.
- Table 2. Histamine concentrations and bacterial counts in tuna loins stored at 3-4°C and abused at 20°C [histamine (mg kg⁻¹) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets].
- Table 3. Histamine concentrations and microbial counts in fresh and frozen tuna loins stored at 0-2°C and abused at 30°C [histamine (mg kg⁻¹) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets].
- Table 4. Histamine concentrations and microbial counts in fresh and frozen tuna loins stored at 6-7°C and abused at 30°C [histamine (mg kg⁻¹) in average and standard error in brackets, bacterial counts (CFU gr⁻¹) in average of log₁₀ and standard error in brackets].
- Table 5. Bacterial Isolates and histamine concentration in culture supernatants after incubation at 37°C for 48 h.
- Fig 1. Regression analysis of histamine and Aerobic Colony Counts, Enterobacteriaceae and Coliforms