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Report on Pulsed Light Processing of Seafood

Confidential to: Sea Fish Industry Authority

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Summary

Pulsed light has been proposed as a technique for surface pasteurisation of food surfaces. Materials to be treated are exposed to multiple short flashes (typically 300µs) of a broad spectrum (200 to 1100nm), high intensity, white light in order to inactivate micro-organisms on the sample surface. The aim of this project was to assess the potential for the use of pulsed light to extend the shelf-life of seafood by reducing the microbial loading on the product surface. Trials were undertaken to establish if pulsed light could reduce the level of naturally present organisms including *Pseudomonas* on raw cod, smoked salmon slices and cooked whelks. Further trials explored the inactivation of *Listeria innocua* inoculated onto the surface of cooked cockles, cooked and picked crab meat, smoked salmon slices and cooked whelks.

The levels of naturally occurring *Pseudomonads* and Total Viable Counts (TVC) in whelks and cod were not substantially reduced by the pulsed light treatment. The levels of inoculated *Listeria innocua* in the cockles and crab were not substantially reduced by the pulsed light treatment.

Smoked salmon showed a maximum reduction of up to 3.6 logs in the level of *Pseudomonas* after treatment and an average log reduction of 1.3 in the level of TVC's after pulsed light treatment. The level of inoculated *L. innocua* on the surface of smoked salmon was reduced by 1.8 log cycles.

Product changes were observed after the pulsed light treatment in some of the species tested. Cod in particular had a markedly increased "fishy" odour after the pulsed light treatment. This odour was identified by Solid Phase Micro Extraction (SPME) as trimethylamine and

was greatly increased after the pulsed light treatment at some conditions. It should be noted however that very severe treatment conditions were employed – the focus of the experiments was to demonstrate microbiological inactivation not to optimise the process with respect to maintaining product quality, this work would need to be conducted within the framework of a much larger project.

A second stage of trials was undertaken using smoked salmon and observed the effect of the pulsed light treatment over the shelf-life on the levels of Enterobacteriaceae and lactic acid bacteria. No Enterobacteriaceae were observed over the shelf-life of the product in any of the treated and untreated samples. The levels of lactic acid bacteria over the shelf-life were variable; treated samples generally had lower levels of lactic acid bacteria, however the results were too variable to conclude that this was definitely a treatment effect. There was also no detectable difference between the treated and untreated samples over the shelf-life when analysed for taints by SPME.

Overall pulsed light treatment had no effect on the microbial population on the surface of cod, whelks, cockles or crab which has been suggested is due to the surface morphology of these products. A reduction was seen with smoked salmon as the surface is smoother and decontamination using pulsed light could be of commercial significance and is worthy of further exploration in this product.

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1.0 Introduction

Pulsed light treatment is a novel method for the decontamination of surfaces and has been proposed as a technique for surface pasteurisation and sterilisation of food and food contact surfaces. The process involves flashing surfaces with a broad spectrum white light which has been reported to inactivate microorganisms. The broad spectrum white light produced by pulsed light equipment is typically in the wavelength 200-1100nm which comprises of 15% ultraviolet light, 50% visible light and 35% near infrared light (personal communication, Claranor SA). During the pulse, the system delivers a spectrum that is 50,000 more intense that sunlight at the earth's surface (personal communication, Claranor SA). The system provides very short pulses (300µs) to the surface of the test material. This short pulse reportedly heats the surface of the material to approximately 160°C during the moment of the flash but this is a very short duration (300µs) so it is essentially a non-thermal process. The mode of action on microorganisms is thought to be due to the UV part of the broad spectrum light. Studies in which the UV component was filtered out showed marked reductions in process efficacy (Rowan et al. 1999). The UV light has a photochemical and photothermal effect on the microbial cell. The primary target for UV at these wavelengths is nucleic acids and several mechanisms are thought to underlie this inactivation including chemical modifications and DNA cleavage. The UV light transforms the pyrimidine bases in the DNA and forms dimers so the microorganism cannot replicate (Sharma and Demirci, 2003). Under certain experimental conditions with continuous UV treatment, repair of the damaged DNA can occur, this repair does not occur after pulsed light treatment (Elmnasser *et al*. 2007). Commercial systems are currently in operation for continuous sterilisation of packaging components, at rates of up to 40,000 per hour.

CCFRA has a 4 lamp, pulsed light system (Figure 1) and a high fluence 2 lamp system (Figure 2) on loan from Claranor SA, a French company specialising in pulsed light applications. Both units are laboratory-scale batch systems but the technology is fully scalable to production size, continuous processing.

There appears to be very little data available in the public domain regarding the efficacy of pulsed light processing for the treatment of seafood. As fish is a highly perishable product. Any process that extends the saleable life of fish would be of benefit to the fish supplier and retailer as it could lead to a reduced wastage and higher quality at point of sale.

Figure 1 Four lamp pulsed light system (image courtesy of Claranor SA)

Figure 2 High fluence two lamp pulsed light system
(Image courtesy of Claranor SA)

2.0 Materials and Methods

2.1 Pulsed light treatment

Pulsed light studies were carried out on cod, cockles, crab, smoked salmon and whelks. All samples apart from inoculation studies with *Pseudomonas* on cod loin were treated in a high fluence pulsed light system as shown in figure 2. This system had two lamps positioned at the top and an adjustable platform to move the sample closer to the lamp.

The inoculation studies previously mentioned with *Pseudomonas* on cod loin were carried out in the four lamp system shown in figure 1. In all cases, all of the samples were flashed as close as possible to the lamps. The treatment conditions for each of the products are shown in table 1 for the high fluence two-lamp unit and table 2 for the four-lamp system.

Table 1 Treatment conditions for each of the products tested on the high fluence two-lamp system

Product type	Distance from lamp	Voltage	Number of pulses (per side)	Comments
Cod	5cm	3000	5 pulses	Treated on both sides
Cockles	1.5cm	3000	5 pulses	Treated on both sides
Crab	1.5cm	3000	5 pulses	
Smoked salmon	1cm	3000	5 pulses	Treated on both sides
Whelks	3cm	3000	5 pulses	Treated on both sides

Table 2 Treatment conditions for the product tested on the four-lamp system

Product type	Top Lamp	Right hand lamp	Left hand lamp	Bottom lamp	Voltage	Number of pulses	Comments
Cod	3cm	8cm	8.5cm	10.5cm	3000	20	20 flashes applied to each side of fish. A total treatment of 40 flashes.

Samples of cod in the high fluence system were not tested as close to the lamp as was possible, this was because when the sample of cod was treated at 2.5cm (i.e. as close as possible to lamp) for five pulses, the sample started to smoke and a very fishy odour was present. The lamp was therefore lowered to 5 cm, this decreased the presence of the odour. It was decided to test the samples to identify the odour being produced. An untreated control, a sample which had been treated at 2.5cm from the lamp for 3 pulses (both sides) and a sample which was treated at 5cm for 5 pulses (both sides) were sent for SPME analysis (see section 2.31).

Fluence reading taken at 5cm from the lamp using a Gentec laser power and energy meter (Gentec,USA) were on average 1.61 J/cm ^{2} (sd= 0.05, n= 3). Readings at the other product testing heights (1.5cm and 1cm) were not possible because the fluence meter was thicker than the distance between product and lamp, the best estimate for fluence is therefore that the treatments were in excess of 1.6 J/cm^2 with a likely maximum of 2.9 J/cm^2 (likely upper limit estimate by Claranor, SA).

Samples were place on the treatment platform and raised to the required height. The sample was flashed and the unit was opened. The sample was removed with, sterile forceps and the sample platform was decontaminated with alcohol wipes. The sample was turned over, raised to the required height, and flashed again (in all samples apart from the crab). The samples were removed with forceps and placed in a sterile stomacher bag and microbiological analysis carried out.

Samples of cod, whelks and smoked salmon were photographed using a digital imaging system (Digieye, UK) and the resultant images are shown in appendix 1.0.

2.2 Microbiological studies

2.21 Inoculation studies for Pseudomonas on cod loin

A sample of cod was purchased from a local retailer and cut into 5cm squares. Prior to inoculation the *Pseudomonas* cultures (*Pseudomonas putida*, CCFRA 14211 and *Pseudomonas fluorescens*, CCFRA 4897 and 15937) were grown in 10 ml Nutrient broth (Oxoid, CM1) (NB) incubated at 25°C±1°C for 48 hours. Each 5 cm^2 sample of cod was inoculated with 0.1ml of the *Pseudomonas* inoculum and stored overnight at 5°C overnight

before treatment. Triplicate treated and untreated samples were stored at 8°C and were tested on days 0, 1, 3 and 6 after processing.

Treated samples and controls were weighed out aseptically and a 1:1 dilution was prepared using Maximum Recovery Diluent (MRD)(LabM Lab 103). The samples were stomached and a 2-ml sub-sample was added to 8ml of MRD, to give a 1:10 dilution. A decimal dilution series was prepared using MRD. The spread plate technique was used for the enumeration of *Pseudomonas* using 0.5ml and 0.1ml spread plates on pre-poured plates of *Pseudomonas* Selective Agar Base (PSAB)(LabM Lab108;X108). The plates were allowed to dry, inverted and incubated at 25±1°C for 48±4h, and all typical colonies counted.

2.22 Microbiological method for naturally contaminated products

Smoked salmon and whelks were purchased from a local retailer, cod loin was provided by an industrial partner. Cod and smoked salmon were cut up into five and four cm squares respectively. Whelks were treated singularly. Samples were treated according to the conditions shown in table 1. Pulsed light treated samples and untreated controls were analysed in duplicate for Total Viable Count (TVC) and *Pseudomonads*. Analysis consisted of both surface swab enumeration and enumeration of the macerated sample. For enumeration of the macerated samples, the entire sample was weighed out aseptically and a 1:10 dilution was prepared using Maximum Recovery Diluent (MRD)(Lab M LAB103). The samples were stomached for 1 minute. A decimal dilution series was prepared using MRD. Pour plates (1ml) were used for TVC and 0.5ml and 0.1ml spread plates were used for the enumeration of *Pseudomonas*.

For surface enumeration, duplicate treated and untreated samples were assessed for TVC and *Pseudomonads* using the swab technique. A sterile swab was pre-moistened in MRD and the top and bottom surface of the product being tested was swabbed over its entire surface.

For the enumeration of TVC using both the stomached and swabbed samples, Plate Count Agar (PCA) (LabM Lab 149) was used. The plates were allowed to set, inverted and incubated at 30°C ± 1°C for 48 hours, and all the resultant colonies counted.

For the enumeration of *Pseudomonads*, pre-poured plates of *Pseudomonads* Selective Agar Base was used (PSAB) (LAB M, Lab 108; X108). The plates were allowed to dry, inverted and incubated at 25 ± 1°C for 48 ± 4 hours, and all the typical colonies counted.

2.23 Microbiological method for Listeria innocua inoculated samples

Listeria inoculation studies were carried out on cockles, smoked salmon whelks and crab meat. Cockles, salmon and whelks were purchased from a local supplier. Crab meat was provided by an industrial supplier. Sample sizes tested were as follows:

Crab meat (10g)
Cockles (6-8 cockles)
Smoked Salmon (4cm^2)
Whelks (one whelk)

Three different strains of *Listeria* were inoculated onto the product surface in each case. The strains used were *Listeria innocua* NCTC 11288, *Listeria innocua* CRA 3379 and *Listeria innocua* CRA 7767. Prior to inoculation the *Listeria* cultures were grown in Tryptone Soya

Broth (TSB, Oxoid CM0129) at 30°C ± 1°C for 48 hours. A microscopic count was performed on each culture. The cultures were mixed into a cocktail for inoculation.

Samples of white crab meat (approximately 10g) were inoculated with 0.1ml of the *Listeria* cocktail. Cockles were inoculated with 0.01ml of *Listeria* cocktail on the top and bottom surface. Smoked salmon and whelks were inoculated with 0.1ml of the *Listeria* cocktail distributed over the top and bottom surfaces.

Duplicate treated and untreated samples of smoked salmon and whelks were enumerated for the presence of *Listeria* by both maceration of the whole sample and by swabbing. For practical reasons crab and cockles could not be enumerated for *Listeria* using the swab method. Four samples of treated and untreated crab and cockles treated were therefore enumerated for the presence of *Listeria* using bulk maceration only.

Sample preparation and enumeration using bulk maceration and swabbing was as previously described in section 2.22 but only spread plates were employed. Pre-poured plates of *Listeria* Selective Agar Oxford formulation (OXFORD) (Oxoid CM856 and SR140) were used. The plates were allowed to dry, inverted and incubated at 30°C ±1°C for 48 ±4 hours, and all typical colonies counted.

2.3 Chemical analysis

2.31 Extraction of volatiles by headspace solid phase micro extraction (SPME)

As discussed in section 2.1, cod samples were noted to have an increased 'fishy' odour after treatment very close to the pulsed light lamp source. As a result, samples were treated further

from the lamps. In order to explore this effect, some cod samples (as detailed in section 2.1) were tested for headspace volatiles using SPME.

A four gram sample was placed into a 20 ml vial and sealed. The vial was equilibrated at 75°C for 15 minutes with agitation. The headspace of the vial was then sampled for 15 minutes at 75°C with agitation using a carboxen/polydimethylsiloxane coated SPME fibre. The volatiles adsorbed onto the fibre were analysed by thermal desorption at 300°C in the injector port of a GC/MS. The analysis of volatiles was carried out on a Varian 3800 gas chromatograph (GC) and Varian 320 triple quad mass spectrometer (MS) via a CTC Combi-Pal autosampler. The GC/MS conditions were as follows:

Column: 60m x 0.25mm fused silica with VF-5MS stationary phase
Helium carrier gas flow rate: 1.5ml. Min^{-1}
Desorption temperature: 280°C
Column temperature: 40°C for 1 minute, then 40°C to 250°C at 5°C. Min^{-1}, then 250°c to 350°C at 15°C Min^{-1}, then held for 8 minutes
MS analysis mode: Scan 33-350 *m/z*

Peaks were tentatively identified by spectral matching with the NIST library of mass spectral data. This determined what was present in the fish. It was then interpreted to determine whether any significant difference was present, and whether the treatment of fish had caused any changes.

2.4 Shelf-life studies on smoked salmon

Shelf life studies were conducted on pulsed light treated smoked salmon. As this was a deviation from the original project plans this was agreed with the Seafish project manager before the trials commenced. Four centimeter squares of smoked salmon slices were used as per the experiments described in section 2.22 and 2.23. Untreated control samples were packed into vacuum bags (The Vacuum Pouch Company Ltd, UK) and vacuum sealed in a Multivac vacuum sealing machine (Multivac). Samples were treated in the high fluence pulsed light machine at 3000V at a distance of 1cm from the lamp. Pieces were flashed 5 times and the square removed. The sample platform was wiped with an alcohol wipe to decontaminate the surface and the square of smoked salmon was turned over and flashed a further 5 times. Each sample was placed in a clean vacuum bag and vacuum sealed. The samples were stored between 0-2°C over the shelf-life. Samples were analysed for microbial growth and for SPME analysis on days 0, 4, 6, 8 and 11. Duplicate treated and untreated samples were tested for Enterobacteriaceae and lactic acid bacteria on each sampling occasion. These organisms were considered appropriate because the smoked salmon was stored under vacuum for the shelf-life trial. The swabbing method of enumeration was employed because more consistent results were obtained in previous trials as detailed in section 2.2. Methods were as described in section 2.22.

For enumeration of the Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBGA, Lab M Lab 88) was used with a 1 ml pour plate technique. The plates were allowed to set, overlayed with VRBGA, allowed to set once again, inverted and incubated at 37°C ±1°C for 24 ±2hours, after which all resultant typical colonies were counted.

For enumeration of presumptive Lactic Acid Bacteria, de Man Rogosa Sharpe Agar (MRSA, LabM Lab 93) was used with a 1 ml pour plate technique. Once set the plates were overlayed, allowed to dry, inverted and incubated at 30°C ±1°C for 5 days, after which time all resultant colonies were counted.

3.0 Results

3.1 Microbiology results

3.11 Inoculated *Pseudomonads* on cod

A 0.23 log cfu/g reduction in the levels of *Pseudomonas* was achieved in cod after pulsed light treatment as shown in figure 3. In summary differences in *Pseudomonas* counts between treated and untreated samples over shelf life was negligible. This is likely to be due to surface morphology and shadowing effects.

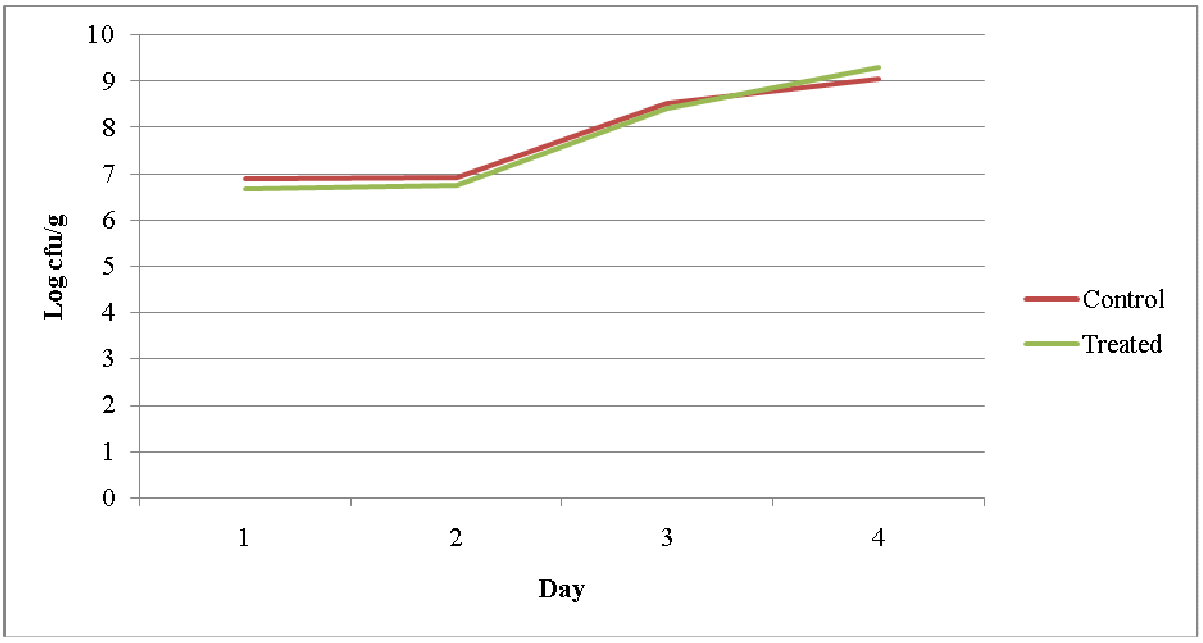


Figure 3 initial *Pseudomonas* inoculated cod at chilled conditions over time with and without pulsed light treatment

3.12 Treatment of products using natural microbial loading

Microbiology results from the samples of un-inoculated cod, whelks and smoked salmon are given in tables 3-5. As can be seen in table 3 there was a 0.53 and 0.66 log cfu/g reduction in the level of TVC in the macerated treated cod and a 0.25 and a 0.65 log reduction when the cod was swabbed. The reduction in *Pseudomonas* level ranged between 0.14 and 0.34 using both swab and maceration methods. In summary, no meaningful reduction were observed.

As shown in Table 4 there was a TVC log reduction of between 1.29 and 1.45 cfu/g in treated smoked salmon when macerated and between 1.03 and 1.24 log reduction when swabbed. The reduction (log cfu/g) in *Pseudomonas* level ranged between 2.88 and 3.63 when macerated and 1.90 log cfu/g when samples were swabbed.

There was no meaningful reduction of TVC or *Pseudomonas* in the treated whelks (Table 5).

Table 3 Reductions of naturally occurring TVC and *Pseudomonads* on cod with and without pulsed light treatment

Total Viable Count cfu/g or cfu/swab						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	7.90E+03		Stomached	3.20E+03	3.51	-0.53
	1.40E+04			2.40E+03	3.38	-0.66
Mean	**1.10E+04**	**4.04**	**Mean**	**2.80E+03**	**3.44**	**-0.6**
Swabbed	6.5E+02		Swabbed	2.50E+02	2.40	-0.65
	1.60E+03			6.30E+02	2.80	-0.25
Mean	**1.13E+03**	**3.05**	**Mean**	**4.40E+02**	**2.64**	**-0.41**
***Pseudomonas* cfu/g**						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	8.10E+03		Stomached	3.90E+03	3.59	-0.34
	9.00E+03			6.20E+03	3.79	-0.14
Mean	**8.55E+03**	**3.93**	**Mean**	**5.05E+03**	**3.70**	**-0.23**
Swabbed	6.90E+02		Swabbed	3.80E+02	2.58	-0.24
	6.40E+02			4.30E+02	2.63	-0.19
Mean	**6.65E+02**	**2.82**	**Mean**	**4.05E+02**	**2.60**	-0.22

Table 4 Reductions of naturally occurring TVC and *Pseudomonads* on smoked salmon with and without pulsed light treatment

Total Viable Count cfu/g or cfu/swab						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	2.60E+05		Stomached	7.40E+03	3.87	-1.42
	1.30E+05			1.00E+04	4.00	-1.29
Mean	**1.95E+05**	**5.29**	**Mean**	**8.70E+03**	**3.93**	**-1.36**
Swabbed	3.30E+03		Swabbed	3.10E+02	2.49	-1.03
	3.30E+03			1.90E+02	2.28	-1.24
Mean	**3.30E+03**	**3.52**	**Mean**	**2.50E+02**	**2.40**	**-1.12**
***Pseudomonas* cfu/g**						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	3.70E+04		Stomached	4.00E+01	1.60	-2.88
	2.30E+04			7.07E+00	0.85	-3.63
Mean	**3.00E+04**	**4.48**	**Mean**	**2.35E+01**	**1.37**	**-3.11**
Swabbed	2.00E+01		Swabbed	7.07E+00	0.85	-1.90
	1.10E+03			7.07E+00	0.85	-1.90
Mean	**5.60E+02**	**2.75**	**Mean**	**7.07E+00**	**0.85**	-1.90

Table 5 Reductions of naturally occurring TVC and *Pseudomonads* on whelks with and without pulsed light treatment

Total Viable Count cfu/g						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	3.30E+04		Stomached	8.20E+04	4.91	0.30
	5.00E+04			9.90E+03	4.00	-0.62
Mean	**4.15E+04**	**4.62**	**Mean**	**4.60E+04**	**4.66**	**0.04**
Swabbed	1.70E+03		Swabbed	3.50E+03	3.54	0.29
	1.90E+03			1.80E+03	3.26	0.00
Mean	**1.80E+03**	**3.26**	**Mean**	**2.65E+03**	**3.42**	**0.16**
***Pseudomonas* cfu/g**						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	1.80E+02		Stomached	1.70E+02	2.23	-0.41
	7.00E+02			7.00E+02	2.85	0.20
Mean	**4.40E+02**	**2.64**	**Mean**	**4.35E+02**	**2.64**	**0.00**
Swabbed	2.00E+01		Swabbed	7.07E+00	0.85	-0.28
	7.07E+00			7.07E+00	0.85	-0.28
Mean	**1.35E+01**	**1.13**	**Mean**	**7.07E+00**	**0.85**	-0.28

3.13 Results from *Listeria* inoculated products

There was between a 0.23 and 0.67 log reduction of *Listeria* on cockles when pulsed light treated (Table 6). Log reductions in crab ranged from 0.05 to 0.24 (Table 7) i.e. no meaningful reduction were observed in either case. In smoked salmon, reduction in *Listeria* levels (log cfu/g) ranged between 0.38 and 0.57 in macerated samples and 1.83 when swabbed (Table 8). There was no meaningful reduction in *Listeria* levels in pulsed light treated whelks (Table 9).

Table 6 Reductions of *Listeria* inoculated on cockles with and without pulsed light treatment (macerated only due to sample shape)

	***Listeria* cfu/g**				
	Controls	**Log**	**Treated**	**Log**	**Reduction**
	1.30E+03		2.80E+02	2.45	-0.45
	7.30E+02		2.00E+02	2.30	-0.60
	6.30E+02		4.60E+02	2.66	-0.23
	4.90E+02		1.70E+02	2.23	-0.67
Mean	**7.88E+02**	**2.90**	**2.78E+02**	**2.44**	**-0.46**

Table 7 Reductions of *Listeria* inoculated on crab with and without pulsed light treatment (macreated only due to sample shape)

	***Listeria* cfu/g**				
	Controls	**Log**	**Treated**	**Log**	**Reduction**
	4.80E+02		3.60E+02	2.56	-0.24
	6.10E+02		4.90E+02	2.69	-0.11
	6.80E+02		5.60E+02	2.75	-0.05
	7.50E+02		7.90E+02	2.90	0.10
Mean	**6.30E+02**	**2.80**	**5.50E+02**	**2.74**	**-0.06**

Table 8 Reductions of *Listeria* inoculated on smoked salmon with and without pulsed light treatment

***Listeria* cfu/g or cfu/swab**						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	2.90E+03		Stomached	2.60E+03	3.41	-0.37
	9.60E+03			1.70E+03	3.23	-0.57
Mean	**6.25E+03**	**3.80**	**Mean**	**2.15E+03**	**3.33**	**-0.47**
Swabbed	1.90E+03		Swabbed	2.00E+01	1.30	-1.83
	8.00E+02			2.00E+01	1.30	-1.83
Mean	**1.35E+03**	**3.13**	**Mean**	**2.00E+01**	**1.30**	**-1.83**

Table 9 Reductions of *Listeria* inoculated on whelks with and without pulsed light treatment

***Listeria* cfu/g or cfu/swab**						
Control		**Log**	**Treated**		**Log**	**Reduction**
Stomached	6.00E+02		Stomached	2.60E+03	3.41	0.07
	3.80E+03			1.70E+03	3.23	-0.11
Mean	**2.20E+03**	**3.34**	**Mean**	**2.15E+03**	**3.33**	**-0.01**
Swabbed	1.20E+02		Swabbed	1.00E+02	2.00	-0.47
	4.70E+03			2.80E+02	2.45	-0.02
Mean	**2.95E+02**	**2.47**	**Mean**	**1.90E+02**	**2.28**	**-0.19**

3.2 Chemical analysis

3.21 Results of SPME analysis

The likely cause of the 'fishy' taint observed in the pulsed light treated cod samples was identified as trimethylamine. This is produced from the degradation of trimethylamine oxide, it is a natural process in fish, but can be accelerated in adverse conditions. As well as trimethylamine, another compound was found that could not be more specifically identified other than as an amine. This amine is also likely to cause 'fishy' taints, and is likely that it developed in the fish in a similar manner to trimethylamine. The levels of trimethylamine and the unidentified amine in the pulsed light treated samples were higher than in the control sample (Figure 4), the levels of these compounds were highest in the sample flashed 5 times at 5cm in the pulsed light system. It should again be stressed that the aim of these studies

was to demonstrate a microbiological effect not to optimise the process. 'Severe' processing conditions were therefore employed in order to have the best possible chance of seeing an anti-microbial effect. It is possible that where microbial kill was observed, this could be achieved using a milder pulsed light treatment, perhaps with a reduced impact on product quality changes.

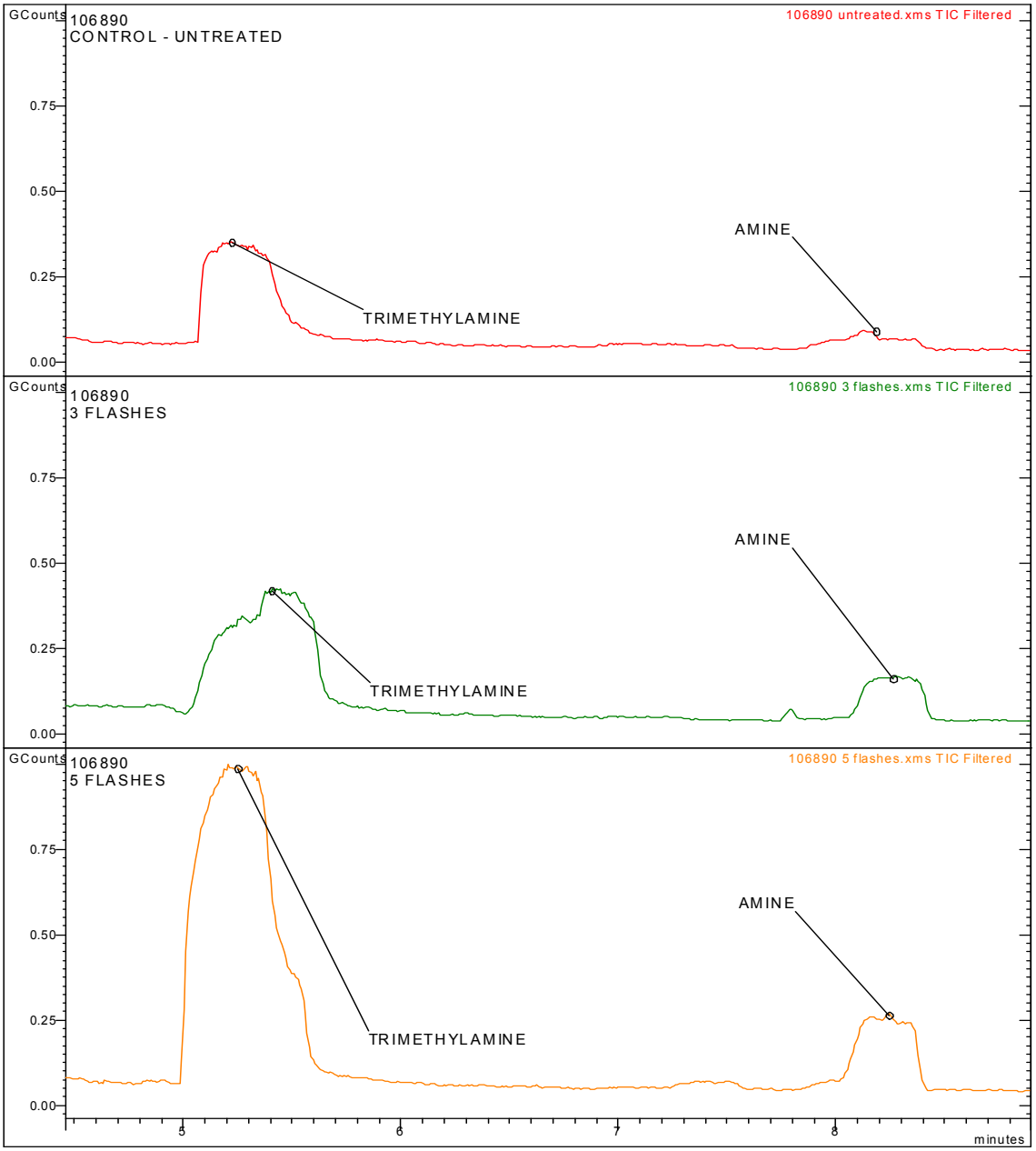


Figure 4: Chromatograms obtained by SPME/GC/MS analysis of the control and two pulsed light treated samples (3 flashes and 5 flashes): detail of amine peaks

3.3 Results from shelf-life trials

The results from the shelf-life trial on un-inoculated smoked salmon are shown in table 10. There were no Enterobacteriaceae present in any of the treated samples or controls. Levels of lactic acid bacteria, were variable throughout the storage trial. One treated replicate generally had a lower level of lactic acid bacteria present than the other replicate and this could be due to a reduction caused by the pulsed light treatment, it could also however be due simply to raw material variation. The results were too variable to conclude that the lower levels of lactic acid bacteria in one sample was a treatment effect and further studies would be required to establish this. It should be noted that even after 11 days of storage lactic acid bacteria were non-detectable in some samples. This *suggests* a positive pulsed light effect which might be improved with process optimisation. The results from the SPME volatile headspace analysis picked up no substantial differences between the treated and the untreated sample over the shelf-life of the smoked salmon.

Table 10 Reductions of Enterobacteriaceae and Lactic Acid Bacteria over storage on pulsed light treated smoked salmon

	Presumptive Enterobacteriaceae		Lactic Acid Bacteria	
	Untreated	Treated	Untreated	Treated
Day 0	<10	<10	3.10E+02	6.00E+01
	<10	<10	2.20E+02	<10
Day 4	<10	<10	5.80E+02	<10
	<10	<10	4.00E+01	1.60E+03
Day 6	<10	<10	1.70E+02	3.00E+01
	<10	<10	2.50E+02	<10
Day 8	<10	<10	3.00E+01	3.00E+01
	<10	<10	1.00E+01	<10
Day 11	<10	<10	1.80E+02	3.60E+02
	<10	<10	1.20E+03	<10

4.0 Conclusions

The levels of naturally occurring *Pseudomonads* and TVC were measured before and after pulsed light treatment in cod and whelks. The levels of inoculated *Listeria innocua* were measured in cooked and picked white crab meat and whelks before and after pulsed light treatment. There was no meaningful reduction in numbers of organisms in any of these products for any of the microbiological tests performed by either the swabbing or stomaching method employed. The process was not optimised for these products, and it is known for example, that there is up to a 50% reduction in fluence two centimetres away from the centre of the lamp in the high fluence system. When interpreting the results it should be recognised that improvements may be possible with process optimisation. In cod, detrimental effects of pulsed light treatment were identified in the form of taints but this could potentially be avoided by the use of less severe pulsed light treatments.

In the original work plan, it was intended that the first stage of the project would observe the effect of pulsed light treatment on cod, whelks and cooked picked white crab meat. Because the results suggested no potential benefit of pulsed light for those products tested, additional products were included in the first stage work, specifically, smoked salmon and cockles were added to the scope.

Cockles and the smoked salmon were inoculated with *Listeria innocua* and the smoked salmon was also tested for naturally occurring *Pseudomonas* and TVC's. Cockles showed no meaningful log reductions in microbial counts after pulsed light treatment, however an effect of the pulsed light treatment on the levels of microorganisms in smoked salmon was observed. The largest log reduction achieved was 3.63 in naturally occurring *Pseudomonas* and a 1.8 log reduction of the marker organism *Listeria innocua* was achieved on the surface

of smoked salmon. These results are similar but slightly higher than that other published results. Ozer and Demirci (2006) achieved a maximum of 1.02 log reductions of *Listeria monocytogenes* on the surface of salmon fillets with a pulsed light treatment of 60 seconds at 8cm from the lamp. The reason an effect of pulsed light treatment was seen in smoked salmon compared to the other products might be due to the surface properties of the product. The surface of the smoked salmon was smoother than the other products and of a more uniform size and shape therefore shadowing effects (which would reduce the effectiveness of the process) on the pulsed light could have been decreased.

Shelf-life trial considered the natural microorganisms present on smoked salmon, results were too variable to establish a conclusive effect of the pulsed light treatment but a reduction in lactic acid bacteria was suggested. It should be noted that even after 11 days of storage lactic acid bacteria were non-detectable in some samples. This *suggests* a positive pulsed light effect which might be improved with process optimisation. There was no detectable difference between the treated and untreated samples over the shelf-life when analysed for taints by SPME.

Overall pulsed light treatment had no effect on the microbial population on the surface of cod, whelks, cockles or crab which has been suggested is due to the surface morphology of these products. A reduction was seen with smoked salmon as the surface is smoother and decontamination using pulsed light could be of commercial significance and is worthy of further exploration in this product.

5.0 Acknowledgements

The provision of cod fillets crab meat from our industrial partners is gratefully acknowledged. The loan of the equipment from Claranor, SA is gratefully acknowledged.

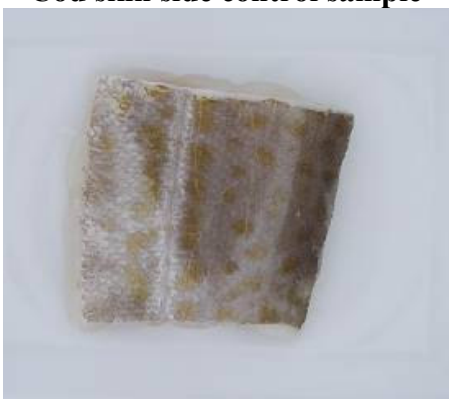
6.0 Appendix 1

Cod flesh side pulsed light treated sample

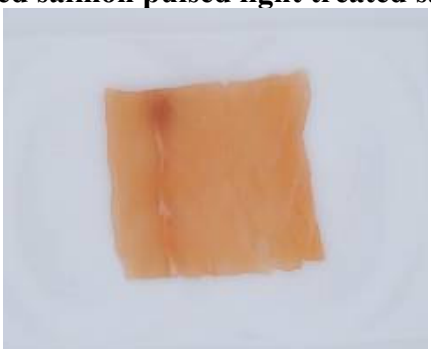
Cod skin side pulsed light treated sample

Cod flesh side control sample

Cod skin side control sample



Smoked salmon pulsed light treated sample



Smoked salmon control sample

Whelks pulsed light treated sample

Whelks control sample

7.0 References

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