Not to be quoted without prior reference to the authors

Fisheries Research Services Report No 03/00

A COMPARISON OF DOMOIC ACID CONCENTRATIONS IN SCALLOP (*Pecten maximus*) GONADS PROCESSED BY FRS ML WITH THOSE PREPARED BY A COMMERCIAL PROCESSOR

P Hess, N A Brown, L A Bates, J J Turriff,
F G Howard and C F Moffat

September 2000

Fisheries Research Services
Marine Laboratory
Victoria Road
Aberdeen AB11 9DB

A COMPARISON OF DOMOIC ACID CONCENTRATIONS IN SCALLOP (*Pecten maximus*) GONADS PROCESSED BY FRS ML WITH THOSE PREPARED BY A COMMERCIAL PROCESSOR

P Hess, N A Brown, L A Bates, J J Turriff, F G Howard and C F Moffat

FRS Marine Laboratory
Aberdeen

SUMMARY

A total of approximately 300 scallops were collected, by commercial charter, from three geographical boxes in the South Minches (SM 10, 11 and 12) on 11 September 2000. At that time the boxes were closed under a Food and Environment Protection Act 1985 (FEPA) Order. The samples were landed at Mallaig under the supervision of an Environmental Health Officer (EHO) from Lochaber District, Highland Council. The scallops were transferred to the Fisheries Research Services Marine Laboratory (FRS ML) in Aberdeen. At FRS ML, under the supervision of an EHO from Aberdeen, they were separated into batches of 12 animals. Three batches of scallops from each of the three sampling boxes were processed by FRS ML and another three batches from each box were processed by Pecten Max, Macduff. This gave a total of 18 sub-samples. Several EHOs were involved in the regulation of these procedures. All samples of gonads were homogenised at FRS ML using routine procedures. To ensure that the FRS ML analyst could not differentiate between samples processed at FRS ML and those prepared by the commercial processor, each sub-sample was given a code by the Aberdeen EHO. The concentration of domoic acid was determined for each of the 18 sub-samples by high performance liquid chromatography (HPLC) with diode array detection. The resulting data was submitted to the Aberdeen EHO who then provided FRS ML with the key to allow sample identification. The data was grouped according to box and processor and a mean concentration determined. All values were greater than the action limit of 20 µg domoic acid/g gonad. The mean results for commercially processed shellfish were slightly higher than those prepared by FRS ML for areas SM 10 and SM 12, and lower than those processed by FRS ML for area SM 11. The differences were not, however, statistically significant. The conclusion is that there was no significant difference in toxin levels between scallops prepared by the commercial processor and those processed by FRS ML.

1. INTRODUCTION

Domoic acid (DA) is the principle compound of the amnesic shellfish group of toxins commonly known as amnesic shellfish poisons (ASPs). The occurrence of DA in shellfish from Scottish waters has resulted in a number of issues being raised by both the Shellfish Industry and MSPs. One such issue concerns the methods used to extract the gonad (roe) from scallops (*Pecten maximus*) and the possibility that the procedure used at the Fisheries Research Services Marine Laboratory (FRS ML) gives higher values for domoic acid, than those obtained from commercially processed scallops. At a meeting with representatives of the Food Standards Agency Scotland (FSAS), MSPs and FRS ML on Tuesday 5 September 2000 it was proposed by MSPs that a study be undertaken to tackle this question. That afternoon, Alasdair Morrison, Deputy Minister for Highlands and Islands and

Gaelic, announced to the Industry, relevant Ministers and the media that an investigation into possible differences in the processing of scallops, and the subsequent toxin levels determined, would be undertaken. It was proposed that the results would be available within one month.

2. COLLECTION OF SCALLOPS

FRS ML arranged for a fishing vessel, *Reul A'Chuain* OB915, to obtain shellfish from a closed area. Dispensation for fishing within an area closed by a FEPA Order (West Coast No 2) was received from the FSAS. The vessel was chartered for 11 September 2000 and asked to obtain scallops from three boxes in the South Minches (Fig. 1). The coordinates for the starting position of each trawl were:

SM10: 56°59.14'N 06°30.88'W
SM11: 56°51.90'N 06°11.90'W
SM12: 56°55.60'N 05°56.40'W

The samples were landed in Mallaig, on the west coast of Scotland, on 11 September 2000. FRS ML, under the supervision of Ms F Wright, Lochaber District, Highland Council EHO, took receipt of the samples which were subsequently transported to Aberdeen, in a temperature controlled environment. The samples arrived at FRS ML in Aberdeen on 11 September 2000. The scallops were maintained at 5°C until Tuesday 12 September 2000.

3. SUB-SAMPLING OF SCALLOPS

On Tuesday 12 September 2000, the bags of scallops were opened under the supervision of Andrea Carson, an Aberdeen City Council EHO. The scallops from each of the three geographical boxes were randomly divided into six batches each of 12 scallops, producing 18 sub-samples in total. Each of the 18 sub-samples was placed in a polyethylene bag and sealed by the EHO with a numbered tag (Table 1). The EHO retained the information on the origin of the individual sub-samples. Three of the sub-samples from each area were selected at random for processing at a commercial processor, the other three remaining at FRS ML for processing by scientific staff. Both lots were stored in the chilled store at 5°C until 13 September 2000.

4. ISOLATION OF THE GONADS

4.1 Commercial Processing

On Wednesday 13 September 2000 the nine sub-samples (three from each sampling box) selected for commercial processing were transported, on ice by FRS ML staff, to Pecten Max, Macduff Industrial Zone, Macduff. The Aberdeenshire Council EHO, Alistair MacBain, broke open the sealed bags, supervised the processing of scallops and ensured that the numbered tag stayed with the correct sample. The dissection process was carried out as follows:

> Each individual scallop was opened by inserting a knife at the hinge and by exercising a small cut between the flat side of the shell and the adductor muscle. The shell was then pulled open by hand and the flat shell half discarded. The mantle was removed prior to making a second cut, between the round half of the shell and the adductor muscle, which allowed removal of the remaining soft parts of the scallop from the second half of the shell. The remainder was placed onto the dissection table and two cuts isolated the white flesh (adductor muscle) and the roe (gonads) from the rest of the soft parts of the scallop. Any dark parts adhering to the adductor muscle were removed by scraping a knife along the white flesh. The adductor muscle and gonad from the 12 individuals which comprised a sub-sample were combined in a fresh bag, together with the numbered tag. The bag was heat-sealed and then placed on ice.

After the heat-sealing of the bags, it was noted by the FRS ML observer and the EHO that the product had not been washed with tap water, as is the normal procedure in the commercial process.

4.2 Processing at FRS ML

On Wednesday 13 September 2000 the nine sub-samples to be processed at FRS ML were dissected in the usual way under supervision of an Aberdeen City Council EHO (A Carson).

> Each individual scallop was opened by inserting a knife at the hinge and by exercising a small cut between the flat side of the shell and the adductor muscle. The shell was then pulled open by hand and the flat shell half discarded. At this stage the scallop was washed with tap water to remove sand and any fluids from organs that might have been cut when opening the shell. The gonads were removed by hand or using a knife and placed in a plastic container.

5. HOMOGENISATION OF THE GONADS

The homogenisation of the gonads for all sub-samples was conducted at FRS ML under the supervision of an Aberdeen City Council EHO. For the industrially processed scallops, the bags were opened and the gonad separated from the adductor muscle. The product was then washed prior to homogenisation, since this step had not been carried out at the processing plant. Each batch of 12 gonads was then homogenised using a Mini-chopper®, which allows the homogenisation of small sample volumes. The mixture was transferred to a plastic pot.

6. CODING OF SAMPLES

To ensure that the analyst could not differentiate between the samples that had been processed commercially and those processed by FRS ML, each pot was labelled by the EHO with a unique code (FSA Samples – A to R; Table 2). Subsequently, the FRS ML analyst assigned the samples a United Kingdom Accreditation Service (UKAS) number as part of the quality assurance procedure (Table 2).

7. DETERMINATION OF TOXIN CONCENTRATION

The samples were analysed using the FRS ML standard procedure M1875 (Confirmatory Method for the Determination of Amnesic Shellfish Toxins by Diode-Array[1]). This method had previously undergone critical evaluation by auditors from UKAS, and was accepted for accreditation. This method comprises of the following steps:

- SOP 1840 (Extraction of Amnesic Shellfish Toxins from Shellfish Flesh HPLC-Determination);
- SOP 1845 (Clean-up of Shellfish Extracts for the HPLC-Determination of Amnesic Shellfish Toxins); and
- SOP 1860 (Determination of Domoic Acid by HPLC/UV/Diode Array).

The extraction and clean-up were carried out on 13 September 2000. The HPLC analysis was performed on a continuous basis during 14 and 15 September 2000.

8. QUALITY CONTROL

On 15 September 2000, a FRS ML deputy technical manager carried out a quality control check. All the samples were analysed as a single batch (Batch 1000). The paperwork associated with this batch can be viewed in FRS' UKAS archive. A laboratory reference material and a method blank were analysed alongside the other samples. The system suitability check, the laboratory reference material (LRM) and the method blank all complied with the acceptance criteria set for this analysis. Four calibration curves were run, one at the start of the samples and three during the analysis of the samples. All four calibration curves complied with the acceptance criteria (linearity from $r^2 > 0.999$, response factor RSD = 1.4%). A calibration standard was analysed at the end as a final systems check. The within batch reproducibility for the LRM was 1.8%. Within batch reproducibility for samples was 0.5 to 8.6%. Therefore, only differences between samples greater than 10% can possibly be interpreted as differences due to handling of the samples.

9. RESULTS

9.1 Observations Relating to the Removal of the Scallop Gonad

The objective of the processing at commercial plants is to remove the edible parts including adductor muscle and roe, whereas the dissection procedure at FRS ML is undertaken to remove only the roe. The commercial processor generally washes the product after all individual samples are shucked. This was omitted by the processor but carried out by a FRS analyst for these samples. Whereas operators at FRS ML wash each individual scallop directly after opening and prior to the dissection process. Further differences in processing may exist when considering the number of commercial operators in Scotland, and the possibility of differing practices at the various processing plants.

9.2 Concentration of Domoic Acid in the Scallop Gonads

The concentration of domoic acid was above the 20 µg/g threshold level for all samples (Table 2). Individual batch concentrations ranged from 21.7 µg/g to 53.3 µg/g.

9.3 Assignment of Data to Samples

After the sample identification key was supplied by the EHO, the data was grouped according to both box number and processor (Table 3). The mean results for commercially processed shellfish were slightly higher than those prepared by FRS ML for areas SM 10 and SM 12, and slightly lower than those dissected by FRS ML for area SM 11.

10. STATISTICAL SIGNIFICANCE

Statistical analysis was carried out to test whether the slight differences observed between the three means were significant (Annex 1). Initially, each of the three areas was considered separately as toxin concentrations may differ significantly due to differences in geographic distribution. When the data were normally distributed and both the commercial set and the FRS ML set had equal variances, a t-test was carried out (Sigmastat v 2.01, SPSS Inc). When data sets have different variances, the t-test is inappropriate. That was the case for the results for SM 12. For these results, a Mann-Whitney Rank Sum–test was carried out. The statistical tests carried out showed that the observed differences in the mean values were not statistically significant.

The ability of the above tests to detect any difference that may exist between the samples processed at FRS ML and the commercially processed samples is somewhat limited. An analysis of variance (ANOVA) gives an improved ability to detect differences due to different processing methods by combining all the data. An additional advantage of using ANOVA is that it takes into account any differences in toxic concentrations between areas. The ANOVA (Annex 1) also gave no evidence of any difference between the commercially processed samples and those prepared at FRS ML.

11. CONCLUSION

Analysis of scallop gonads for DA resulted in no significant difference in the quantity of toxin detected from scallops processed by FRS ML compared to scallops processed by the commercial processors.

12. REFERENCES

1. Quilliam, M.A., Xie, M. and Hardstaff, W.R. 1995. Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood. *J. AOAC International*, **78**(2), 543-554.

13. ACKNOWLEDGEMENT

The authors would like to thank the crew of the *Reul A'Chuain*, the staff of Pecten Max, Macduff and the various EHOs for their assistance and cooperation during this study.

TABLE 1

Details of EHO Tag numbers for each sub-sample. These were assigned prior to selection for processing.

Box	Sub-Sample	EHO Tag
SM 10	1	005319
	2	005327
	3	005355
	4	005431
	5	005433
	6	005455
SM 11	1	005311
	2	005324
	3	005346
	4	005360
	5	005442
	6	005750
SM 12	1	005361
	2	005371
	3	005385
	4	005395
	5	005405
	6	005489

TABLE 2

Concentration of domoic acid (DA) in scallop gonads. This data-set was compiled and submitted to both the FSAS and an EHO in Aberdeen for verification. At this stage staff at FRS ML had no knowledge of which samples had been commercially processed and which had been processed at FRS ML. The EHO sample identifiers (SID) and the FRS ML UKAS SID are also presented.

UKAS SID	EHO SID	DA [µg/g]
8129	FSA SAMPLES - A	30.5
8130	FSA SAMPLES - B	43.9
8131	FSA SAMPLES - C	36.5
8132	FSA SAMPLES - D	26.2
8133	FSA SAMPLES - E	31.3
8134	FSA SAMPLES - F	53.3
8135	FSA SAMPLES - G	34.9
8136	FSA SAMPLES - H	31.7
8137	FSA SAMPLES - I	25.9
8138	FSA SAMPLES - J	32.0
8139	FSA SAMPLES - K	24.8
8140	FSA SAMPLES - L	39.8
8141	FSA SAMPLES - M	36.9
8142	FSA SAMPLES - N	31.3
8143	FSA SAMPLES - O	27.6
8144	FSA SAMPLES - P	38.2
8145	FSA SAMPLES - Q	26.3
8146	FSA SAMPLES - R	21.7

TABLE 3

Domoic acid (DA) concentrations in 18 sub-samples of scallop gonads. The data was grouped according to sampling location and processor after applying the sample identification key supplied by the controlling EHO in Aberdeen.

SM-Area	Processor	EHO Tag	EHO SID	DA [µg/g]
10	Commercial	005319	FSA SAMPLES - E	31.3
10	Commercial	005431	FSA SAMPLES - L	39.8
10	Commercial	005327	FSA SAMPLES - P	38.2
			Mean	**36.4**
10	FRS ML	005355	FSA SAMPLES - C	36.5
10	FRS ML	005455	FSA SAMPLES - D	26.2
10	FRS ML	005433	FSA SAMPLES - N	31.3
			Mean	**31.3**
11	Commercial	005360	FSA SAMPLES - G	34.9
11	Commercial	005324	FSA SAMPLES - H	31.7
11	Commercial	005442	FSA SAMPLES - R	21.7
			Mean	**29.4**
11	FRS ML	005346	FSA SAMPLES - F	53.3
11	FRS ML	005311	FSA SAMPLES - J	32.0
11	FRS ML	005750	FSA SAMPLES - Q	26.3
			Mean	**37.2**
12	Commercial	005385	FSA SAMPLES - A	30.5
12	Commercial	005395	FSA SAMPLES - B	43.9
12	Commercial	005371	FSA SAMPLES - M	36.9
			Mean	**37.1**
12	FRS ML	005489	FSA SAMPLES - I	25.9
12	FRS ML	005405	FSA SAMPLES - K	24.8
12	FRS ML	005361	FSA SAMPLES - O	27.6
			Mean	**26.1**

Figure 1 Sampling area: South Minches, West Coast of Scotland.

							O9	O10	O11	O12	O13
			NM1	NM2	NM3	NM4	O17	O18	O19	O20	O21
			NM5	NM6	NM7	NM8	O25	O26	O27	O28	O29
H2	H3	NM9	NM10	NM11				M1	M2	M3	M4
H5	H6	NM12	NM13	NM14			M8	M9	M10	M11	M12
H8	NM15	NM16	NM17	NM18			M16	M17	M18	M19	M20
H10	NM19	NM20	NM21				M24	M25	M26	M27	M28
SM1	SM2	SM3	SM4								E1
SM5	SM6	SM7	SM8							E5	E6
SM9	SM10	SM11	SM12							E10	E11
SM13	SM14	SM15	SM16							E15	E16
	J1	J2	J3						E20	E21	E22
	J4	J5	J6						E26	E27	E28
	J7	J8	J9	C1	C2					E32	E33
	J10	J11	J12	C3	C4						
	J13	J14	J15	C5	C6						
			C7	C8							
			IS1	IS2	IS3						

Annex 1

Summary of the Statistical Analyses

Individual Box Comparison

Data source: **ASP results for SM 10** (comparison of scallop processing).

Normality Test:	Passed	(P > 0.200)
Equal Variance Test:	Passed	(P = 0.767)

Group Name	N	Mean	Std Dev*	SEM*
Com - SM10	3	36.4	4.5	2.6
FRS - SM10	3	31.3	5.1	3.0

Difference of Means: 5.1.

t = 1.289 with 4 degrees of freedom (P = 0.267).

95 percent confidence interval for difference of means: -5.9 to 16.1.

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.267).

*Std Dev, Standard Deviation; SEM, Standard Error of the Mean.

Data source: **ASP results for SM 11** (comparison of scallop processing).

Normality Test:	Passed	(P > 0.200)
Equal Variance Test:	Passed	(P = 0.503)

Group Name	N	Mean	Std Dev	SEM
Com - SM11	3	29.4	6.9	4.0
FRS - SM11	3	37.2	14.2	8.2

Difference of Means: -7.8.

t = -0.851 with 4 degrees of freedom. (P = 0.443).

95 percent confidence interval for difference of means: -33.1 to 17.6.

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.443).

Data source: **ASP results for SM 12** (comparison of scallop processing)

Normality Test:	Passed	(P > 0.200)
Equal Variance Test:	*Failed*	(P = 0.006)

Due to failure of the equal variance test the customary t-test was not appropriate. Instead the Mann-Whitney Rank Test was undertaken.

Group	N	Median
Com - SM12	3	36.9
FRS - SM12	3	25.9

T = 15.000 n(small)= 3 n(big)= 3 P(est.)= 0.081 P(exact)= 0.100.

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.100).

Analysis of Variance (ANOVA)

Data source: **DA concentrations for all 18 samples from the three boxes and different processors** (comparison of scallop processing).

Difference of Means: 2.8.

f = 0.61 on 1 and 12 degrees of freedom (P = 0.451).

95 percent confidence intervals for difference of means: -5.1 to 10.7.