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Analytical problems in mercury analysis of seafood

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Summary. - It is generally accepted that seafood represents one of the major sources of mercury to man. In this work two interlaboratory proficiency tests are described for the analysis of mercury in seafood. Thirty-seven public control and food industries laboratories participated in the first test, while 29 participants were included in the second one. Moreover, in order to clarify whether sampling of different edible muscle tissues of the same fish could affect the analytical results, the top, the central and the bottom portion of 28 fishes were examined. The different portions of fish showed no significant difference in mercury concentrations. Two different wet digestion methods (microwave oven and reflux in quartz vessels) were also tested in the case of 11 fishes. A systematic difference was observed between the two sets of results obtained with these digestion methods.

Key words: seafood, mercury, interlaboratory proficiency test, microwave digestion, reflux digestion.

Riassunto *(Problemi analitici nell'analisi del mercurio nei prodotti ittici).* - I prodotti della pesca rappresentano una delle maggiori fonti di esposizione a mercurio per l'uomo. In questo lavoro vengono descritti due esercizi di intercalibrazione per l'analisi del mercurio in prodotti ittici. Hanno partecipato ai due test rispettivamente 37 e 29 laboratori di istituzioni pubbliche di controllo e di industrie alimentari. Per chiarire se il campionamento di parti differenti della frazione edibile dello stesso pesce possa influire sul risultato analitico sono state inoltre analizzate le porzioni di testa, centrale e di coda di 28 pesci. Non è stata evidenziata alcuna differenza significativa nella concentrazione di mercurio tra le diverse frazioni. Sono stati anche confrontati due differenti metodi di mineralizzazione (forno a microonde e riscaldamento a riflusso in recipienti di quarzo) su 11 pesci. Tra i gruppi di dati ottenuti con i due differenti metodi di mineralizzazione è stata rilevata una differenza significativa.

Parole chiave: prodotti ittici, mercurio, circuiti di intercalibrazione, mineralizzazione con forno a microonde, mineralizzazione a riflusso.

Introduction

Quality of analytical data produced by routine laboratory work is a very important issue which deserves the highest attention from the laboratory management. This is particularly so when the results have an impact onto public health. The increasing availability of certified reference materials (CRMs) and the more and more frequent participation in proficiency testing programmes have substantially improved reliability of analytical data even if all this required a remarkable effort by routine laboratories.

Given the scenario mentioned above it would be interesting to evaluate the activity undertaken in a public health laboratory which, as a part of its own activity, has to control the Hg content in more than 800 seafood samples yearly. In order to assess the state-of-the-art of the Italian official method and to evaluate reliability of results of Hg analysis in seafood, two interlaboratory proficiency tests were organized by the Italian Association of Public Health Chemists (Unione Italiana Chimici Igienisti, UICI) and the Italian Association for Quality Controls (Associazione Italiana Controllo di Qualità, AICQ) [1].

For the first of them, held in 1988, mussel and tuna fish samples were distributed to 41 laboratories, 37 of which supplied the results. Figs 1 and 2 show the good level of analytical results (SD is only ± 0.071 and ± 0.118 mg/kg in the two cases, respectively), uncertainty is approximately of ± 0.1 mg/kg.

In the second test, held in 1990, the number of participating laboratories was 38, 29 of which supplied results. All of them received tuna fish samples highly contaminated with Hg. Fig. 3 shows the results of this second test. By comparing standard deviations in the three cases it is self-evident that the third is worse than the other two, probably due to the fact that the participating laboratories were not so expert as for the first exercise (only 9 laboratories participated in both tests).

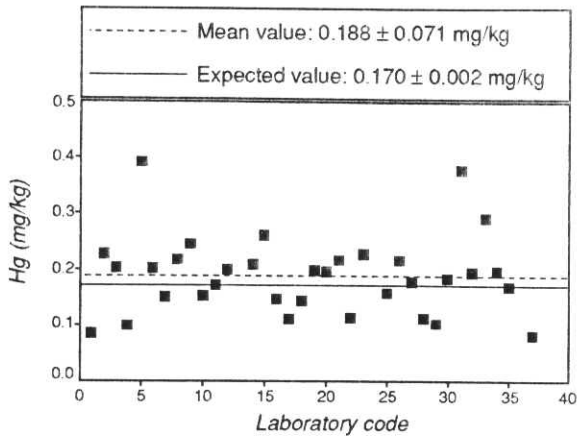

Fig. 1. - Results of the first intercomparison of analyses of Hg in mussels.

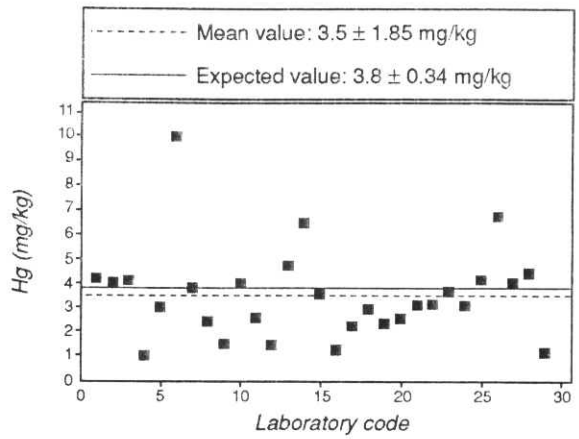

Fig. 3. - Results of the second intercomparison of analyses of Hg in tuna fish.

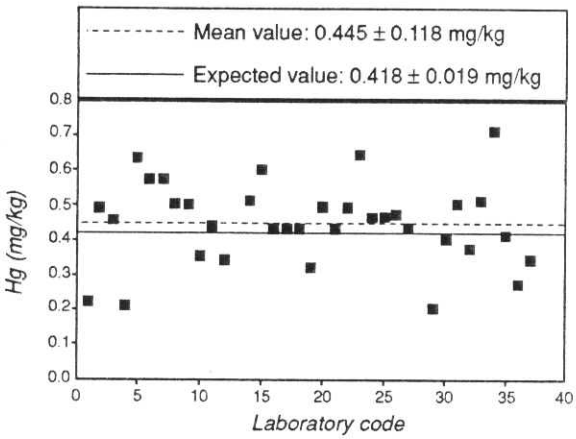

Fig. 2. - Results of the first intercomparison of analyses of Hg in tuna fish.

For both programmes samples were prepared by the Joint Research Center of the European Commission, in Ispra Establishment, and the Hg contents were determined by neutron activation analysis (NAA). Results thus obtained were taken as expected values.

Evidence achieved so far confirms that to improve analytical data quality it is mandatory to participate frequently in proficiency tests. The question then arises of how it is possible to reconcile the amount of work necessary to perform such tests with the routine work in a laboratory that analyzes yearly more than 10,000 samples of water, food, waste material, drugs, etc. The reliable performance of Hg analyses on more than 800 seafood samples yearly is in conflict with the need of carring out determinations as fast as possible because fresh fish needs to be sold immediately. This goal is definitely to be reached since exceeding the limit of 0.5 mg/kg involves the rejection of seafood products with the ensuing economic damage to fishing activities. The Venice laboratory data show that ca. 5% of the samples analyzed exceed the mentioned limit.

In recognition of the fact that there are some critical steps affecting analytical results for food samples, attention was given to the part of fish body where sampling, and on time requirements of two different wet digestion methods. In order to verify whether different sampling sites can influence analytical results, the top and the bottom (excluding head and tail) as well as the central portion of the body of same fish were considered. Twenty-eight fishes of different weight, length and body burden of Hg were selected and analyzed.

Materials and methods

To prepare the samples, non-edible parts were excized from fresh fishes, and the remaining dorsal muscle tissues subdivided into head side, central and tail side portions. Specimens were separately homogenized in a blender.

Two different wet-digestion methods were used: a) digestion of homogenized samples (5 g) in quartz vessels with 20 ml concentrated HNO_3 for 3 h under reflux. The digestion solution was then treated for 1 h with 5 ml H_2O_2 (35% m/v) under reflux; b) digestion of homogenized sample (0.5 g) with 5 ml concentrated HNO_3 in a microwave apparatus (Milestone mod. MLS 1200). The following instrumental parameters were selected: step 1, 25 % power for 3 min; step 2, 35% power for 1 min; step 3, 40% power for 1 min. After cooling sample were added with 1 ml H_2O_2 (35% m/v) and digested again as described above.

Final detection was made by AAS (Perkin-Elmer 2100, USA) by resorting to the cold vapour system FIAS 200 (carrier solution, 3% HCl and reducing agent 0.1% $NaBH_4$ in 0.05% NAOH). Calibration was done with standard solutions of $Hg(NO_3)_2$ in 1% HNO_3 (standard addition method).

Recovery was 95% for digestion under reflux and 98% for digestion with the microwave system.

Table 1. - Mercury concentration (mg/kg) in different sampling sites (head, centre and tail) of the 28 fishes considered

	Weight (kg)	Length (cm)	Head side Hg (mg/kg)	Centre Hg (mg/kg)	Tail side Hg (mg/kg)
1	10.0	85	0.41	0.35	0.32
2	1.0	60	0.18	0.19	0.20
3	5.0	106	1.44	1.40	1.31
4	20.0	83	0.56	0.59	0.51
5	23.0	150	0.49	0.60	0.44
6	4.0	115	0.72	0.59	0.74
7	9.0	85	2.65	2.98	2.56
8	2.0	95	12.06	12.17	12.75
9	22.0	150	1.25	1.37	1.42
10	2.0	85	19.24	16.69	16.21
11	3.5	130	1.73	1.64	1.74
12	6.5	73	0.53	0.51	0.49
13	6.5	73	0.49	0.61	0.58
14	13.0	180	0.41	0.35	0.35
15	25.0	105	1.04	1.05	1.08
16	11.0	110	0.48	0.50	0.47
17	3.0	125	0.99	0.97	0.94
18	2.8	90	0.78	0.78	0.81
19	3.9	110	0.24	0.20	0.22
20	4.6	135	2.01	1.81	1.78
21	1.5	75	0.75	0.75	0.78
22	5.5	150	1.11	1.21	1.24
23	2.8	120	0.66	0.56	0.53
24	1.1	105	0.72	0.66	0.68
25	2.3	91	1.00	1.10	1.24
26	29.5	232	0.76	0.73	0.67
27	8.0	120	0.98	0.94	0.96
28	2.3	80	0.94	0.86	0.84

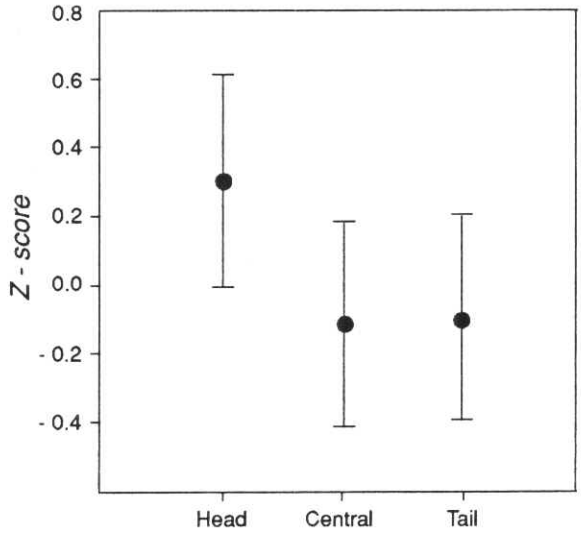

Fig. 4. - Mean and standard deviation of Hg concentration (normalized values) in the different sampling sites (both sides and centre) of 28 fishes investigated.

Results and discussion

Table 1 reports length, weight and Hg concentration of single portions of edible muscle tissues of various fishes living in Mediterranean Sea. Due to the broad range of concentrations, an immediate comparison of data was not possible. The analysis of variance (portion of fish used as classification factor) had consequently been carried out after a normalization of the results using the Z-scores calculated from $(X_i-X_m)/S$, where X_m and S are, respectively, the mean value and the standard deviation of Hg concentration (X_i) of the three fractions (head side, centre, tail side) of fish. Statistical evaluation of the normalized data confirms that there is no significant variation among the groups considered (Fig. 4).

Table 2 shows the Hg concentration of the different parts of the 11 fishes digested with the two above mentioned methods. The analysis of variance (digestion method as classification factor) of results obtained with the different methods, had been carried out again after normalization of data using Z-scores calculated from $(X_i-X_m)/S$, where X_m and S are, respectively, the mean

Table 2. - Mercury concentration (mg/kg) in different sampling sites of 11 fishes analyzed after two different digestion procedures (R: reflux; M: microwaves)

Procedures	Head side Hg (mg/kg)	Centre Hg (mg/kg)	Tail side Hg (mg/kg)
1-R	0.29	0.24	0.23
1-M	0.41	0.35	0.32
2-R	0.13	0.13	0.18
2-M	0.18	0.19	0.20
3-R	1.05	0.98	0.98
3-M	1.44	1.40	1.31
4-R	0.32	0.29	0.27
4-M	0.56	0.59	0.51
5-R	0.32	0.56	0.40
5-M	0.49	0.60	0.44
6-R	0.49	0.45	0.53
6-M	0.72	0.59	0.74
7-R	1.93	2.04	1.82
7-M	2.65	2.98	2.56
8-R	11.68	10.51	11.46
8-M	12.06	12.17	12.75
9-R	0.86	0.89	0.89
9-M	1.25	1.37	1.42
10-R	17.44	15.44	15.65
10-M	19.24	16.69	16.21
11-R	1.41	1.36	1.45
11-M	1.73	1.64	1.74

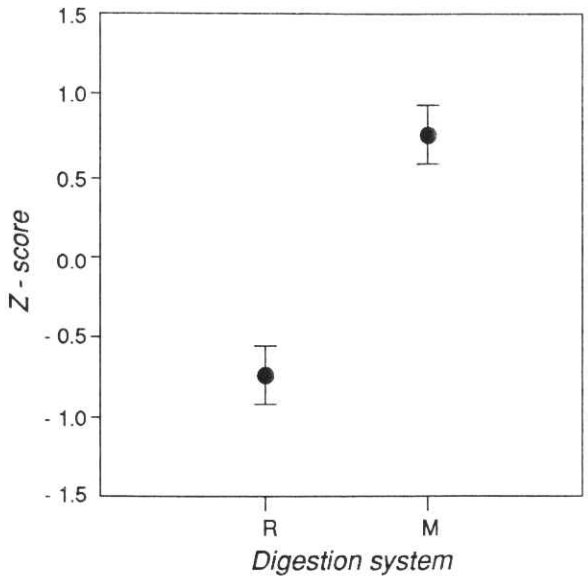

Fig. 5. - Mean and standard deviation of Hg concentration (normalized values) in the different sampling sites of 11 fishes analyzed after two different digestion procedures. R: reflux; M: microwaves.

value and the standard deviation of Hg concentration (X_i) obtained by the two methods. There is a significant difference between the two groups considered ($p<0.01$). The corresponding results are plotted in Fig. 5.

Recovery and accuracy of the two digestion methods were evaluated by determining the Hg content of two CRMs (tuna fishes CRMs no. 463 and 464). The results of five indipendent determinations are shown in Table 3.

It is important to note that there is a systematic difference between the two digestion methods for both fresh and lyophilized samples. The microwave system gives Hg concentrations higher then those obtained by reflux method: + 12% in relation to reference materials and + 30% in relation to single portions of fresh fish. To explain this behaviour two hypothesis are possible, namely: a) the oxidizing properties of HNO_3 and H_2O_2 might be insufficient to destroy completely the organic matrix in the reflux system; b) residual nitrogen oxides from the digestion process could disturb spectrometric measurements, thus leading to a positive bias in the case of samples digested with the microwave system.

In order to improve the accuracy of microwave digestion systems a further digestion cycle with 1 ml of H_2O_2 (35% m/v) was added with the same timing and power parameters of the second one. A 3% of

Table 3. - Mean value of total Hg of two certified reference materials obtained by two different digestion systems

	Microwave M ± SD (mg/kg)		Reflux M ± SD (mg/kg)	Expected value M ± SD (mg/kg)
		P < 0.01		
CRM 463	3.13 ± 0.06	↔	2.79 ± 0.03	2.85 ± 0.16
		P < 0.01		
CRM 464	5.49 ± 0.03	↔	4.93 ± 0.03	5.24 ± 0.10

improvement in results was achieved. Neither doubling the amount of oxidizing agents and reflux time with the quartz vessel method led to appreciable improvements. Even the AOAC method did not bring about significantly better performance [3].

It is very important to remark that the use of the CRMs has permitted a light improvement. In fact, with the further digestion, the Hg concentration determined for CRM 463 and CRM 464 are inner the expected standard deviations.

A very important remarque is the big discrepancy between the data obtained by the two considered systems in the CRM (12%) and in the fresh fish (30%). This is probably depending also by disomogenity of muscle tissues of fresh fishes. A further and necessary study has to be developed to ensure the quality of results of Hg concentration in the tissues of fresh fish.

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