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Food Chemistry 87 (2004) 343–347

Food Chemistry

www.elsevier.com/locate/foodchem

Determination of zinc and copper in fish samples collected from Northeast Atlantic by DPSAV

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Received 15 August 2003; received in revised form 26 November 2003; accepted 26 November 2003

Abstract

Zinc and copper contents in the edible parts (muscle, fillet) of 19 commercially used fish species from Northeast Atlantic (Tampen, North of Shetland Islands, Faroe Islands and Copinsay) were determined by means of DPSAV (differential pulse stripping anodic voltammetry). In the sample preparation step, all frozen fish samples were lyophilised, milled in a ball mill and finally decomposed in an oxygen plasma ashing chamber. Among the fishes from four sampling locations, the highest zinc and copper concentrations were found in small-spotted catshark (*Scyliorhinus caniculus*) and with 8.6 mg/kg for zinc and mackerel (*Scomber scombrus*) and with 0.84 mg/kg for copper, respectively. The accuracy of the concentrations determined in this study was checked by the measurements of the certified reference material CRM No. 422, cod muscle from the Commission of the European Communities, Community Bureau of Reference. All Zn and Cu concentrations observed from species of Northeast Atlantic showed that fish from this area is a good source of these essential elements and the developed method is accepted as a good analytical routine method for these samples.

Keywords: Zinc; Copper; DPSAV; Voltammetry; Plasma asher; Fish

1. Introduction

Zinc and copper are essential elements for human beings (Oehlenschläger, 1997; Tüzen, 2003) which means that they must be a part of our diet. However, these elements also can be toxic at high concentrations. There are numerous sample treatment procedures (Moeller, Ambrose, & Que Hee, 2001; Ranau, Oehlenschläger, & Steinhart, 1999; Yaru, Bainok, & Day, 1999) and several analytical methods described for the determination of these trace elements (Bassari, 1994; Locatelli & Torsi, 2001, 2002; Locatelli, 2003; Roméo, Siau, Sidoumou, & Gnassia-Barelli, 1999; Zauke, Savinov, Ritterhoff, & Savinova, 1999).

Zinc is found in almost every cell and in a wide variety of foods. It is present in seafood in mg/kg amounts and there have been no reports of concentrations in the edible parts of food fish that form a hazard to health. With an average zinc content of 3–5 mg/kg wet fish, it is a good source for this essential element (Oehlenschläger, 2002). The essential role of zinc is based on its roles as an integral part of a number of metalloenzymes and as a catalyst for regulating the activity of specific zinc-dependent enzymes. Molluscs contain the greatest concentration of zinc. Among all food sources of animal origin, oyster is the richest source of zinc. Recorded values in classostreid oysters exceed 4000 mg/kg of dry weight (Lall, 1995).

Copper is required for iron utilization, and as a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue and phospholipids. Numerous studies have focussed on copper metabolism in fish and on toxic effects related to heavy metal pollution in the aquatic environment (Lall, 1995); however, it is not toxic for humans in low concentrations (Linder & Hazegh-Azam, 1996).

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0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.11.018

The aim of this study was to determine zinc and copper contents in the edible parts (muscle, fillet) of 19 commercially used fish species from the Northeast Atlantic and to develop routine analytical method for the determination of zinc and copper using DPSAV, Differential Pulse Stripping Anodic Voltammetry, using an autosampler (Hung, Meng, Han, Chuang, & Huang, 2001; Locatelli, Fabbri, & Torsi, 2001; Nürnberg, 1983; Stoeppler & Nürnberg, 1979).

2. Materials and methods

Nineteen fish species, belonging to 10 families from the Northeast Atlantic, were collected onboard the fishery research vessel, "Walter Herwig III" at 4 fishing grounds (Tampen, North of Shetland Islands, Faroe Islands and Copinsay) in summer 2001.

The fish samples from Northeast Atlantic were immediately frozen after suitable preparation onboard and kept in the deep freezer before analyzing. All frozen samples were lyophilised in a Finn-Aqua Lyovac GT 2 freeze dryer (Parameters: ambient temperature 15–25°C, vacuum 5–10 Pa, duration at least 48 h) and finally finely milled in a ball-mill made from agate (Fritsch, Planetary Ball Mill, pulverisette 5, Idar-Oberstein, Germany). After milling, all samples were kept in high-density polyethylene bags at room temperature in a desiccator until mineralization. Approximately 0.4 g lyophilized sample was weighed into Petri dishes which were put in a closed low temperature microwave-activated oxygen plasma processor (Plasma Prozessor 200-G, Technics Plasma GmbH, München, Germany) for mineralization (Power supply 350–360 W, vacuum 60–90 Pa, oxygen partial pressure $2.0–2.5 \times 10^5$ Pa), duration of decomposition 144–168 h. The decomposed samples were quantitatively transferred into 100 ml volumetric flasks and dissolved in suprapure sulphuric acid (0.2%, w/w) at pH 2.

In this study, DPSAV, i.e. differential pulse stripping anodic voltammetry, (746 VA Trace Analyzer, Metrohm Ltd., Switzerland), with an autosampler (695 VA Autosampler, Metrohm Ltd., Switzerland) was used for the determination of trace elements. The experimental conditions for the determination of zinc and copper by differential pulse stripping anodic voltammetry are shown in Tables 1 and 2.

All solutions were prepared with deionised water obtained from a NANOpure II water purification system (Sybron/Barnstead, Boston, Massachusetts, USA).

Standard zinc and copper solutions were prepared from a Titrisol concentrate containing 1000 mg Zn/l and 1000 mg Cu/l (Zn: Merck No. 109953, Cu: Merck No. 109987, Darmstadt, Germany) with addition of 2 ml $HClO_4$ (70% w/w, suprapure) and filling up to 1000 ml.

Table 1
Experimental conditions for the simultaneous determination of Zn by differential pulse stripping anodic voltammetry (DPSAV)

Deposition potential	−1100 mV
Final potential	−850 mV
Deposition time	15 s
Delay time before potential sweep	10 s
Potential scan rate	13.33 mV/s
Stirring rate	2000 rpm

Supporting electrolyte: HMDE (Hanging Mercury Drop Electrode).

Table 2
Experimental conditions for the simultaneous determination of Cu by differential pulse stripping anodic voltammetry (DPSAV)

Deposition potential	−200 mV
Final potential	−150 mV
Deposition time	60 s
Delay time before potential sweep	10 s
Potential scan rate	13.33 mV/s
Stirring rate	2000 rpm

Supporting electrolyte: HMDE (Hanging Mercury Drop Electrode).

Table 3
Zinc and copper concentrations of standard reference material CRM No: 422 (mg/kg dry weight), analyses were performed in triplicate

CRM No. 422 Cod muscle meat	Certified	Found
	Mean ± SD	Mean ± SD
Zinc	19.6 ± 0.5	15.3 ± 0.4
Copper	1.05 ± 0.07	1.08 ± 0.33

Working solutions were $HClO_4$ (Suprapure, Merck No. 100517) and EDTA ($c = 0.02$ mol/l, Merck No. 159294).

The accuracy of the concentrations determined in this study was checked in triplicate analyse by the measurements of the certified reference material CRM No. 422 cod muscle from the Commission of the European Communities, Community Bureau of Reference. Zinc and copper concentrations of this standard reference material are presented in Table 3. Quevauviller (1995) reported the zinc values determined by the commission as 19.6 ± 0.5 mg/kg. In this study, mean zinc concentration was determined as 15.3 ± 0.4 mg/kg. The copper concentration was reported as 1.05 ± 0.07 mg/kg and it was determined as 1.08 ± 0.33 mg/kg in this study.

3. Results and discussion

The zinc and copper concentrations of the edible part (muscle, fillet) of 19 fish species belonging to 10 families from Northeast Atlantic waters were determined by using DPSAV.

The zinc concentrations of the Northeast Atlantic fishes investigated are given in Table 4. Of all Northeast

Table 4
Zinc concentrations of Northeast Atlantic Fishes (mg/kg w/w)

	Species	*n*	Mean ± SD	Minimum	Maximum
Tampen	Haddock (*Melanogrammus aeglefinus*)	5	2.9 ± 0.211	2.6	3.3
	Cod (*Gadus morhua*)	2	3.3 ± 0.065	3.2	3.3
	Saithe (*Pollachius virens*)	6	3.7 ± 0.085	3.7	3.8
	Ling (*Molva molva*)	3	3.1 ± 0.048	3.1	3.2
	Tusk (*Brosme brosme*)	3	3.2 ± 0.223	3.0	3.5
	Hake (*Merluccius merluccius*)	2	3.3 ± 0.056	3.2	3.3
	Lemon sole (*Microstomus kitt*)	2	2.8 ± 0.101	2.6	2.9
	Megrim (*Lepidorhombus whiffiagonis*)	4	2.5 ± 0.201	2.1	2.9
	Grey gurnard (*Chelidonichthys gurnardus*)	3	3.5 ± 0.389	3.0	4.2
	John dory (*Zeus faber*)	2	3.1 ± 0.028	3.1	3.1
Shetland Islands	Saithe (*Pollachius virens*)	5	4.0 ± 0.110	3.8	4.5
	Anglerfish (*Lophius piscatorius*)	4	3.0 ± 0.033	3.0	3.0
	Ling (*Molva molva*)	3	3.0 ± 0.421	2.7	3.6
Faroe Islands	Whitting (*Merlangius merlangus*)	6	3.0 ± 0.179	2.8	3.4
	Haddock (*Melanogrammus aeglefinus*)	2	2.6 ± 0.241	2.2	3.0
	Anglerfish (*Lophius piscatorius*)	4	3.0 ± 0.264	2.6	3.3
Copinsay	Mackerel (*Scomber scombrus*)	10	4.1 ± 0.602	3.3	5.2
	Haddock (*Melanogrammus aeglefinus*)	4	3.4 ± 0.577	2.5	4.0
	Small-spotted catshark (*Scyliorhinus caniculus*)	1	8.6 ± 0.145	8.5	8.7

Atlantic samples, the highest zinc content was found in the muscle of small-spotted catshark from Copinsay with 8.6 mg/kg, while in the megrim (*Lepidorhombus whiffiagonis*) from Tampen the lowest concentration (2.5 mg/kg) was detected. Samples of Tampen ling (*Molva molva*) had the highest concentration of zinc (3.1 mg/kg) and megrim had the lowest (2.5 mg/kg). For the Shetland Islands, the values were 4.0 mg/kg in saithe (*Pollachius virens*) as maximum and 3.0 in anglerfish (*Lophius piscatorius*) and ling as minimum. For fish from the Faroe Islands, it was found that whiting (*Merlangius merlangus*) and anglerfish exhibited the highest values (3.0 mg/kg), while haddock (*Melanogrammus aeglefinus*) had only 2.6 mg/kg, as the lowest value. In Copinsay, the highest value was 8.6 mg/kg for small-spotted catshark and the lowest value was 3.4 mg/kg for haddock. In samples of the Gadidae family, which presented the most specimens in all samples, saithe from the Shetland Islands had the highest zinc concentration (4.0 mg/kg) and haddock from the Faroe Islands the lowest (2.6 mg/kg). Harms (1975) reported zinc concentrations for cod (*Gadus morhua*) from onshore and offshore waters of German Bight (North Sea) between 2.0 and 7.5 mg/kg. In this study, the mean zinc concentration of cod was 3.3 mg/kg. Cronin, Davies, Newton, Pirie, Topping, and Swan (1998) investigated some fish from North East Atlantic and the range of mean concentrations of zinc to be 2.2–6.7 mg/kg. Mormede and Davies (2001) reported zinc concentrations of fish muscle from 2.62 to 5.53 mg/kg for fish from the North East Atlantic. These values were similar in the present study.

The copper concentrations of the Northeast Atlantic fishes are presented in Table 5. The highest concentration of all samples was found in mackerel from Copinsay with 0.84 mg/kg and the lowest in cod from Tampen, with 0.11 mg/kg. It was also found, for the samples from Tampen, that hake (*Merluccius merluccius*) had highest concentration of copper (0.41 mg/kg) and cod had the lowest (0.11 mg/kg). For the Shetland Islands, the values were 0.58 mg/kg in saithe as maximum and 0.19 mg/kg in ling as minimum. For fish from the Faroe Islands, Cu found to be 0.31 mg/kg in whiting, as highest value, and 0.16 mg/kg in anglerfish as the lowest. In Copinsay, the highest value was 0.84 mg/kg for mackerel and lowest was 0.19 mg/kg for haddock. In samples of the Gadidae family, saithe from the Shetland Islands had the highest copper concentration (0.58 mg/kg) and cod from Tampen the lowest (0.11 mg/kg). Harms (1975) also reported copper concentrations for cod between 0.2 and 1.0 mg/kg. In this study, mean copper concentration of cod was 0.11 mg/kg. Cronin et al. (1998) reported copper concentrations of fish from the North East Atlantic between 0.01 and 0.47 mg/kg. Mormede and Davies (2001) reported copper concentrations of fish muscle between 0.10 and 0.83 mg/kg for fish from the North East Atlantic. These values were between 0.11 and 0.84 mg/kg in this study.

The results showed that the concentrations of, zinc and copper, were higher in Copinsay fish, than in fish from other sampling locations. The mean zinc concentrations for all specimens from Tampen, North of Shetland Islands, Faroe Islands and Copinsay investigated were 3.14 mg/kg wet weight, 3.33 mg/kg wet

Table 5
Copper concentrations of Northeast Atlantic Fishes (mg/kg w/w)

	Species	*n*	Mean ± SD	Minimum	Maximum
Tampen	Haddock (*Melanogrammus aeglefinus*)	5	0.18 ± 0.02	0.14	0.22
	Cod (*Gadus morhua*)	2	0.11 ± 0.01	0.11	0.12
	Saithe (*Pollachius virens*)	6	0.39 ± 0.01	0.38	0.39
	Ling (*Molva molva*)	3	0.13 ± 0.00	0.13	0.13
	Tusk (*Brosme brosme*)	3	0.14 ± 0.02	0.13	0.18
	Hake (*Merluccius merluccius*)	2	0.41 ± 0.29	0.20	0.61
	Lemon sole (*Microstomus kitt*)	2	0.25 ± 0.07	0.19	0.38
	Megrim (*Lepidorhombus whiffiagonis*)	4	0.23 ± 0.11	0.13	0.47
	Grey gurnard (*Chelidonichthys gurnardus*)	3	0.32 ± 0.05	0.23	0.39
	John dory (*Zeus faber*)	2	0.13 ± 0.01	0.12	0.14
Shetland Islands	Saithe (*Pollachius virens*)	5	0.58 ± 0.06	0.46	0.65
	Anglerfish (*Lophius piscatorius*)	4	0.24 ± 0.07	0.19	0.29
	Ling (*Molva molva*)	3	0.19 ± 0.02	0.16	0.22
Faroe Islands	Whitting (*Merlangius merlangus*)	6	0.31 ± 0.06	0.22	0.42
	Haddock (*Melanogrammus aeglefinus*)	2	0.26 ± 0.05	0.21	0.34
	Anglerfish (*Lophius piscatorius*)	4	0.16 ± 0.04	0.10	0.23
Copinsay	Mackerel (*Scomber scombrus*)	10	0.84 ± 0.11	0.70	0.97
	Haddock (*Melanogrammus aeglefinus*)	4	0.19 ± 0.04	0.13	0.25
	Small-spotted catshark (*Scyliorhinus caniculus*)	1	0.51 ± 0.02	0.50	0.53

weight, 2.87 mg/kg wet weight and 5.37 mg/kg wet weight and those of copper were 0.23 mg/kg wet weight, 0.34 mg/kg wet weight, 0.24 mg/kg wet weight and 0.51 mg/kg wet weight, respectively. The recommended daily intakes are 15 mg Zn for adult males and 12 mg Zn for adult females and 1.5–3.0 mg Cu (Anon, 1991).

4. Conclusion

In the present study, the frozen samples were lyophilized, milled and then mineralized in a closed low temperature microwave oxygen plasma processor equipped with a high performance pump plasma asher chamber. Then the mineralized samples were dissolved in 0.2% sulphuric acid and lead and cadmium concentrations were determined by DPSAV, differential pulse stripping anodic voltammetry (by hanging mercury drop electrode), using an autosampler. This configuration is novel for determination of Zn and Cu in fish.

Voltammetry is notable for its very low detection limits and its sensitivity. DPSAV has some important advantages, such as simultaneous determination of up to 4 elements, low contamination risk and high precision (Oehlenschläger, 1993).

This study is of utmost importance since the proposed method simplifies the multi-analyses of zinc and copper in edible parts of fish.

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